Association of dentine hypersensitivity to tooth wear

Olley, Ryan

Awarding institution:
King's College London

KINGS COLLEGE LONDON DENTAL INSTITUTE AT GUY'S, ST THOMAS' AND KING'S COLLEGE HOSPITALS

Association of dentine hypersensitivity to tooth wear

Thesis submitted for degree of Doctor of Philosophy

Ryan C Olley

BDS Bsc (Hons) MJDF RCS Eng AHEA

Department of Prosthodontics and Department of Biomaterials Science

Kings College London Dental Institute

London SE1 9RT

September 2012

To mum

Abstract

Dentine hypersensitivity (DH) affects up to 57% of patients following exposure of un-occluded dentine tubules however the aetiology is incompletely understood. These studies investigated the association of DH to tooth wear. A prevalence study investigated risk factors associated with tooth wear and DH on all tooth surfaces in 350 subjects aged 18-35 in SE England. Sextant cumulative scores for DH and tooth wear were validated and positive correlations existed between both ($p<0.0001$). Two randomised, single blind *in situ* studies investigated the degree of dentine tubule occlusion provided by desensitising dentifrices following four days of twice daily brushing with agitated acid challenges on days three and four. In the first *in situ* study involving 28 healthy subjects, samples were imaged daily using Scanning Electron Microscopy (SEM) and graded using a 'standard' visual ordinal scale. On days one and two, an 8% strontium acetate and 8% arginine based desensitising dentifrice demonstrated more occlusion than control paste ($p<0.0001$) and water ($p<0.0001$, $p=0.0003$). On day four, strontium demonstrated more occlusion than all other treatments ($p<0.0001$). In a second *in situ* study involving 30 subjects, an innovative computerised and imaging method was created and validated to quantify tubule occlusion. Samples were imaged with Tandem Scanning Microscopy (TSM) and then SEM. Intra-class correlation of the number of un-occluded tubules counted visually and then by the computational analysis on 10% (n=47) randomised SEM or TSM images was ≥ 0.8. Positive Spearman correlations existed between the visual ordinal 'standard' and the SEM ($r=0.58$) and TSM ($r=0.42$) computational analyses ($p<0.001$, $n=469$). At day four, the TSM computational analysis and the 'standard' showed that an experimental dentifrice containing 5% calcium sodium phosphosilicate produced more occlusion than controls ($p<0.0001$). These studies refute the null hypothesis that there

is no association between DH, tooth wear and the patency of the dentine tubules. Accurate techniques were developed to measure DH.

Acknowledgements

Professor David Bartlett, my primary supervisor and head of department. I am very grateful for all of his experienced guidance and support both throughout my PhD. It has been thoroughly inspiring and made the process even more enjoyable. I appreciate how quickly he has provided sound feedback on my work and this has helped me complete in good time.

Dr Rebecca Moazzez, my second supervisor. I am grateful for her guidance throughout the PhD and for providing useful additional feedback to my research questions. She has been supportive and I am very appreciative of her input.

Dr Ron Wilson has been a source of inspiration and experience in clinical statistics. Despite a busy work schedule, he has made time to offer me advice and I am thankful for all his help throughout the PhD.

Dr Rupert Austin, my PhD mentor and friend. It has been a pleasure to share the ups and the downs together first as SHOs then as PhD students. He is a good friend and I thank him for his support and advice.

Messrs Peter Pilecki and Richard Mallett have been extremely helpful in my laboratory studies. I am grateful for their friendship and assistance.

Professor Timothy Watson, Head of Research at KCLDI. I am grateful for his ongoing encouragement in my career and for his sound feedback and advice.

Dr Manoharan Andiappan and Professor Nora Donaldson, for their kind advice on some statistical analysis.

Dr Richard Foxton for his advice and support in my PhD.

Dr Jose Rodriguez for his sound advice and friendship throughout my PhD.

Dr Charles Parkinson, Mr Nathan Hughes and Dr Steve Mason at GlaxoSmithKline. GSK funded the clinical studies and provided useful advice and feedback on my research. Also, Professor Nicola West for her advice on the prevalence study and Dr Fred Festy for his advice on Image J software.

The subjects and sites of the clinical studies for their cooperation.

Dr Garrit Koller and Dr Richard Cook both for their kind suggestions.

Philip Semple, Rupert Austin, Priya Lankathilake, Obi Egbuniwe and Janet Liao for proof reading this thesis.

Mr John Woodcock and the dental technical team for their ongoing support and friendship throughout my undergraduate and postgraduate training.

To my friends who have supported and encouraged me along; Janet, Nichola, Priya K, Priya L, Kath, Eddie, Harry, Peter, Gabriel, Obi, Andy, Phil, Malhar, *et al.*

And finally to my mum, Jane for the excellent upbringing she provided and for her unparalleled support and interest in my work.

Preface

The first chapter is a review of the literature focusing on dentine hypersensitivity (DH), tooth wear and measurement techniques. The second chapter is a validation of the methodology used in chapters 3, 4 and 5. Part of chapter two, section 1 has been presented orally at an international conference. The third chapter is a prevalence study involving DH and tooth wear measurement on 350 subjects, which was run in collaboration with Professor Nicola West at Bristol Dental Institute and was part of a wider European Prevalence study. The fourth chapter is an *in situ* study investigating the properties of dentifrices designed to treat DH. This is a published study and has also been presented in a poster at a national conference. The fifth study is an *in situ* study comparing methods to measure dentine tubule occlusion of an experimental dentifrice. It has been presented orally at an international conference. The clinical studies were partly funded by GlaxoSmithKline.

I confirm this is my work.

Unless stated, all statistical analysis was performed using STATA® 11 software (StataCorp. Texas, USA).

Glossary of abbreviations

BEWE	Basic Erosive Wear Examination
CSLM	Confocal Scanning Laser Microscopes
DH	Dentine Hypersensitivity
EDX	Energy Dispersive X-ray Spectroscopy
ESEM	Environmental Scanning Electron Microscopy
FIB-SEM	Focused Ion Beam-Scanning Electron Microscopy
IQR	Inter-quartile range
KCLDI	Kings College London Dental Institute
NA	Numerical Aperture
NCCL	Non Carious Cervical Lesion
RDA	Relative Dentine Abrasivity
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
TSM	Tandem Scanning Microscopy
TWI	Tooth Wear Index
VEDE	Visual Erosion Dental Examination
WHO	World Health Organisation

Table of contents

Table of tables

Table of figures

Chapter 1 Review of the literature

1.1 Dentine Hypersensitivity (DH) terminology and definition

Dentine hypersensitivity (DH) is a common clinical condition typically characterised by a short, sharp pain affecting the permanent dentition (Addy, 2002). The current definition of DH, first suggested by Dowell *et al.* in 1983 (Dowell and Addy, 1983) and later finalised by an international workshop on the design and conduct of clinical trials for DH (Holland *et al.*, 1997) states that DH 'is characterised by short sharp pain arising from exposed dentine in response to stimuli typically thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other form of defect or pathology'. The first part of this definition is a clinical description of the condition and the second part differentiates it from other clinical conditions, which may have identical symptoms but different management strategies (Dowell *et al.*, 1985). These other clinical conditions might include, for example, dental caries, cracked tooth, post-restorative sensitivity, medication sensitivity or bleaching sensitivity (Addy, 2002).

The definition was more recently modified by the Canadian Advisory Board for Dentine Hypersensitivity who suggested it would be appropriate to substitute 'disease' for 'pathology' (Canadian *et al.*, 2003). This is because 'disease' relates more to the outcome of the condition rather than its cause or effect (or 'pathology'). The Oxford English Dictionary defines disease as 'a disorder of structure or function in a human, animal, or plant, especially one that produces specific symptoms or that affects a specific location and is not simply a direct result of physical injury' (Dictitionary). Despite the international consensus on the above definition, DH is not included in the WHO classification of diseases.

Furthermore, the terminology of DH may not be wholly accurate. Indeed, clinical and histological observation of dentition reveal that teeth exhibiting DH may appear no different from non-sensitive teeth (Seltzer *et al.*, 1963) and therefore other terms such as dentine sensitivity may appear more appropriate considering dentine will only elicit a painful response when stimuli are applied. On the other hand, not all dentine is sensitive which suggests that certain areas of dentine could be more sensitive, or hypersensitive. This has been reviewed (Addy, 1990; 2002; Dababneh *et al.*, 1999; Pashley *et al.*, 2002). Current disparity over the terminology for this condition is caused in part by a historical lack in understanding of the mechanism of DH and subsequently, its aetiology. This has posed problems in arriving at a differential diagnosis and subsequent management strategies, which have often, lead to recurrence of the condition (Dababneh *et al.*, 1999).

I will first explain the accepted mechanism of DH, its prevalence and significance, then discuss possible aetiologies including tooth wear and management strategies. I will then explain currently used methods used to measure DH and tooth wear clinically and in the laboratory. Considering that the term 'dentine hypersensitivity' has been commonly used for decades, the term is adopted in this thesis.

1.1.1 Mechanism of DH

Understanding the mechanism of DH is critical to help our understanding of its aetiology and subsequent management (Dababneh *et al.*, 1999). Historically, three mechanisms were proposed in the aetiology of DH (Hall *et al.*, 2000):

1. Stimulation of nerve endings in dentine,
2. Chemical or electrical stimulation of odontoblasts,

3. Hydrodynamic theory.

The hydrodynamic theory was first proposed in 1900 (Gysi, 1900) and following favourable evidence in the mid-twentieth century to support the Brannstrom hydrodynamic theory over other theories (Brannstrom, 1963) it became widely adopted. It involves a hydrodynamic liquid phase within the dentine tubules and was demonstrated *in vitro* using stimuli to produce fluid flow within patent or exposed dentine tubules, which in turn were suggested to produce excitation of nerves within the pulp (Brannstrom and Johnson, 1978). The theory assumes that dentine tubules are present and patent (or un-occluded) from the surface of dentine to the pulp and supra-gingival. This is supported by studies, which have used techniques to qualify the presence of dentine tubules on the cervical areas of recently extracted sensitive and non sensitive teeth using histology, SEM imaging and dye penetration within dentine tubules (Absi *et al.*, 1987; 1989; Ishikawa, 1969). It has also been demonstrated *in vivo* using replica impression techniques of exposed sensitive dentine, which revealed dentine tubules microscopically (Absi *et al.*, 1989). These various techniques are described in more detail in the section 1.16. In one study (Absi *et al.*, 1987), teeth with sensitivity and no caries scheduled for extraction were imaged using SEM images (at x1000 magnification). The diameter of dentine tubules at the dentine surface was recorded using a granulated eyeglass. The diameter of dentine tubules was almost two times greater in sensitive areas (0.83μm) compared with non-sensitive areas (0.43μm) of the tooth (Absi *et al.*, 1987). It was later shown that that the hydraulic conductance of fluid (or the ease with which fluid can move across a unit surface area under a unit of pressure per unit of time) within dentine is determined by fluid pressure, length of dentine tubules, the viscosity of fluid and most importantly, by the radius of the dentinal tubules raised to the fourth power (Pashley, 1990a; Pashley, 1994). Overall, the

density and diameter of patent dentine tubules at the dentine surface and patency of the dentine tubules to the pulp is proportional to the degree of fluid permeation through dentine tubules and DH. Extrapolating quantitative results on the size and number of dentine tubules and linking this with DH in the *in vivo* situation is difficult, if not impossible.

1.2 Dentine tubules

Dentine tubules represent the congenital pathway of the odontoblasts from the dentine enamel junction (DEJ) to the pulp, tracing a shallow 's' shaped route with minor secondary curvatures. The dentine tubules are surrounded by a thin layer of peritubular dentine, which is highly mineralised, composed mostly of apatite crystals and consists of almost no collagen. The tubules are separated by a matrix of intertubular dentine composed mostly of type I collagen fibrils in a non collagenous ground substance reinforced by apatite. The collagen fibrils are randomly arranged at right angles to the dentinal tubules with the apatite crystals orientated with their long axes parallel to the fibrils. If peritubular dentine is formed within the tubules, it is more accurately termed intratubular dentine. This can obliterate the tubules and results in sclerotic or translucent dentine, which often has a glassy appearance (Ten Cate, 1998).

Variations exist in the number of dentine tubules throughout dentine. Depending on the study, the number of tubules in occlusal dentine ranges from 18, 000- 24, 500 in outer dentine nearest the tooth surface, 27, 600- 40, 400 in middle dentine and 36, 100- 52, 000 in inner dentine closest to the pulp (Fosse *et al.*, 1992; Garberoglio and Brannstrom, 1976; Mjor and Nordahl, 1996; Olsson *et al.*, 1993; Pashley, 1989). The diameter of tubules also varies, from approximately 0.8μm at the dentine-enamel junction at the cervical margin, 1.2μm at the mid-portion of dentine and 2.5μm at the

pulpal surface. The percentage of tubules, intertubular dentine and peritubular dentine vary from 22%- 1%, 12%- 96% and 60%- 3% at the pulp and dentine enamel junction respectively (Marshall *et al.*, 1997).

Secondary dentine refers to dentine, which is laid down slowly after the tooth has fully formed and erupted and slightly decreases the size of the pulp chamber. It often contains an irregular distribution of dentine tubules. 'Tertiary', 'reparative', 'irritation', 'reactionary' or 'acellular' refer to dentine laid down in response to irritation, such as dental caries, and often contains no dentine tubules (Ten Cate, 1998).

1.3 Pulp nerve supply

The dental pulp has a rich sensory nerve supply which originates from the trigeminal nerve. Most of these nerves are nociceptive and communicate pain. At the periphery of the pulp are the nerve endings of the A-delta fibres. These are stimuilated by relatively low threshold stimuli and the speed of action potentials is greater than the other nerve endings. They cause a short sharp pain sensation and are the fibres stimulated in DH. More central within the pulpal chamber are the C-fibres. The C-fibre pain occurs in classic toothache and is indicative of serious inflammation spreading deep into the pulp, which is irreversible. Unlike A-delta pain, it does not disappear after a stimulus is removed and often lingers for minutes. It might also occur spontaneously, without a stimulus. It is therefore outside the remit of class DH, based on the definition in section 1.1. Figure 1 show the location of the main sensory pain receptors in the pulp (Whitworth 2010).

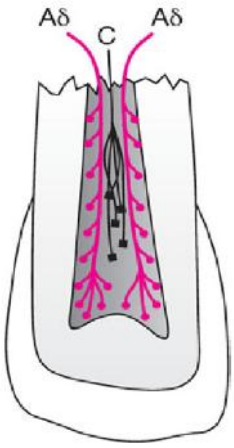


Figure 1 Location of predominant sensory pain receptors in the pulp. A-delta fibres lie peripherally, whereas the c fibres lie centrally

1.4 Prevalence of DH

The reported prevalence of DH is highly variable depending on which study is quoted. It ranges from over 50% in patient reported population studies (BSDA, 2011; Gillam *et al.*, 1999; Kleinberg, 2002). However, in studies involving professional clinical diagnosis, the prevalence and variation is less (approximately 15%, range 8- 30%), (Addy, 2000; 2002; Dababneh *et al.*, 1999; West, 2006). Differences between studies are likely due to different methods used to diagnose DH, variation in the sample population and the setting in which the study was carried out (Que *et al.*, 2010b). It is important to use both patient centred and clinical diagnoses, to ensure that DH is not underreported or unrecognised by clinicians and patients respectively (Boiko *et al.*, 2010). The various techniques used to diagnosis DH clinically will be described in more detail later in section 1.15.2.

DH mainly affects the permanent rather than primary dentition. It may occur on any tooth surface, but has been shown to often occur in the buccal cervical area, perhaps

in association with tooth wear lesions that affect cervical areas of teeth known as Non Carious Cervical lesions (NCCLs) (Addy *et al.*, 1987d; Addy, 2002; Bamise *et al.*, 2008; Flynn *et al.*, 1985; Orchardson and Collins, 1987b; Smith *et al.*, 2008; Vanuspong *et al.*, 2002). DH is also associated with gingival recession (Addy *et al.*, 1987d).

It was shown that DH more commonly affects canines and first premolars, then incisors and second premolars and finally molars (Orchardson and Collins, 1987b). Studies demonstrate some similarities that canine and premolar teeth (Addy *et al.*, 1987d; Fischer *et al.*, 1992; Flynn *et al.*, 1985) or premolar and molar teeth (Rees, 2000) are the most common sites for DH. However, other studies demonstrate a higher prevalence of DH in molars than the other teeth, with upper molars the most commonly affected followed by premolar or canines and then finally incisors (Chabanski and Gillam, 1997; Rees *et al.*, 2003; Rees and Addy, 2004).

In two recent large UK studies using professional clinical diagnoses by trained dentists in practice, the prevalence of DH ranged from 2.8-4.1% (Rees and Addy, 2002; 2004). In the first study, nineteen dental practitioners examined 4, 841 dental patients professionally over a period of one month and 201 patients (4.1%) had symptoms of DH (Rees and Addy, 2002). Patients with DH were aged 30-49 years old with the most commonly affected teeth being upper premolars, followed by upper first molars and lastly incisors. In a later cross sectional study of 5, 477 dental patients attending a general dental practice in the UK during one calendar month, 152 (2.8%) were professionally diagnosed as having DH (Rees and Addy, 2004), which was more common on upper first molars, followed by first premolars, then canines and finally second molars. In both studies, patients who had DH reported that their sensitivity was more commonly associated with cold drinks (rather than hot drinks or tooth brushing). There were also correlations between DH and periodontal disease, smoking and higher

socio-economic groups (Rees and Addy, 2002; 2004). DH was generally more prevalent in young, healthy subjects (Rees and Addy, 2004).

DH may occur iatrogenically and it has been reported to affect up to 57% of the general population in one subject reported study following scaling or root planning (Drisko, 2002). Another study of periodontal patients reported a prevalence of 85- 95% (Chabanski *et al.*, 1996). It had been suggested that in periodontal disease and treatment, bacteria might penetrate into dentine tubules and speculation that sensitivity might occur through a mechanism other than DH (Dababneh *et al.*, 1999). This type of sensitivity was commonly termed root sensitivity. Studies reporting a DH prevalence >30% (Chabanski and Gillam, 1997; Orchardson and Collins, 1987b; Rees *et al.*, 2003) have involved clinical examinations of smaller sample sizes within university hospitals and sample populations selected from the periodontology departments and many of these patients may suffer from more dental or periodontal disease (Que *et al.*, 2010b). Furthermore, periodontal disease often causes loss of molar teeth and it has been suggested (Rees and Addy, 2004) that this might explain the high prevalence of DH amongst canine and premolar teeth in some studies. However, in one study that reported a higher prevalence of DH in molar teeth (Rees and Addy, 2004), the European Federation of Periodontology had not recommended the use of the term root sensitivity to describe sensitivity in association with periodontal disease and treatment. Therefore patients in this study who had periodontal disease and were under-going treatment were included in the remit of DH. It has since been suggested that the low prevalence of DH in this study and others (Rees, 2000; Rees and Addy, 2002; 2004) might be because most subjects were below 50 years of age (Que *et al.*, 2010b).

The demographics of the population such as age and sex are also likely to affect the incidence of DH. For example, it has been shown as being higher in females compared

to males, perhaps due to better oral hygiene in females (Chabanski *et al.*, 1990) or anecdotally based on a suggestion of higher acidic diets in females. The incidence can range from early teenage to 70+ years (Fischer *et al.*, 1992), although other studies report a peak prevalence in age groups 20-25 (Orchardson and Collins, 1987b), 25-29 (Graf and Galasse, 1977), 30-39 (Rees, 2000; Rees and Addy, 2002), 31-40 (Udoye, 2006), 40-45 (Rees *et al.*, 2003), 40-49 (Rees and Addy, 2004) or 50-59 (Liu *et al.*, 1998). Overall, the peak has been around the 20-40 year old age range in reviews (Addy, 2000; 2002; Dababneh *et al.*, 1999; West, 2006), but some studies disagree. In a recent multi-centre and cross sectional study in China, DH was assessed first using a subject reported assessment and then using a clinical assessment of DH and periodontal status in those subjects who reported DH. They examined 2, 640 subjects from community. The recorded prevalence of DH following subject-based assessment was 41.7% and following clinical measurement was 25.5%. The 50- 59 year old age range were more likely to have DH (Que *et al.*, 2010b) perhaps because unlike some previous studies, this study used a balanced age cohort and may have recruited more elderly subjects.

The increased age of DH sufferers may be because the number and severity of tooth wear and periodontal disease increases with aging (Albandar and Kingman, 1999). In addition, it has been reported that the number of restored teeth in younger adults is falling (Nunn *et al.*, 2000) and later suggested that due to less restorative treatment, there may be less reparative dentine formation to protect against DH in adults (Aw *et al.*, 2002). The declining prevalence of DH in subjects over 60 years old (Que *et al.*, 2010b) may be due to the development of secondary or sclerotic dentine (Fischer *et al.*, 1992). For further information on tooth wear, periodontal disease and their association with DH, please refer to sections 1.6 and 1.11.

Pain experienced through DH has been shown to lead to impacts on functional status, eating, drinking, talking, tooth brushing, social interaction, with more subtle effects on emotions and identity (Gibson *et al.*, 2010). Recent research on 280 self-reported DH sufferers supports a link between Oral Health Related Quality of Life and Health Related Quality of Life and DH (Porritt *et al.* 2012). Despite the importance of tooth wear to patients and research to support the prevalence of DH to specific demographics and teeth, there are a lack of clinical observations reporting the underlying aetiological factors involved in DH and the severity of sensitivity.

1.5 Tooth wear

Tooth wear is the irreversible, non-traumatic loss of dental hard tissues due to aetiological processes classified as erosion, attrition, abrasion (Bartlett and Smith, 2000; Ganss and Lussi, 2006) and abfraction (Lee and Eakle, 1984). The World Health Organisation includes attrition, abrasion and erosion in its international classification of diseases (WHO, version 2007). The terms 'tooth wear' and 'tooth surface loss' are interchangeable and the former term will be used in this thesis. The literature increasingly reports that the aetiological processes involved in DH are tooth wear phenomena (Addy, 2002; Dababneh *et al.*, 1999; Markowitz and Pashley, 2008). Also highlighted in these papers is the lack of evidence with regards to the aetiology of DH. I will therefore explain tooth wear and its causes in more detail and then discuss these in association to DH.

Tooth wear may be considered a normal part of aging, or a physiological process, from the anthropological perspective (Whittacker, 2000). Historically, it has been suggested that the human dentition is designed to wear and that this process is important to optimize the functional capabilities of the dentition (Berry and Poole, 1974). Within

dentistry, Smith and Knight first distinguished physiological and active or pathological tooth wear (Smith and Knight, 1984b). Tooth wear may be defined as pathological as opposed as physiological if it appears in relatively younger patients and the rate of progression of tooth wear is fast. In addition pathological tooth wear may threaten tooth survival, cause aesthetic concerns, sensitivity, loss of vitality, failure of restorations or occlusal problems (Al-Omiri *et al.*, 2006; Dahl *et al.*, 1989; Richards *et al.*, 2003; Robb and Smith, 1996; Smith and Knight, 1984b). These are subjective assessments for both the patient and clinician.

Erosion is the loss of tooth surface by chemical dissolution due to an acid, which is not produced by the oral flora, but originates from intrinsic or extrinsic sources (Ten Cate *et al.*, 2008). Intrinsic erosion is caused by stomach acid and arises due to vomiting or gastro-oesophageal reflux disease whereas extrinsic erosion is caused by factors such as diet, lifestyle, environmental factors and some medicaments. Attrition is physical wear as a result of the action of antagonist teeth. Abrasion is physical wear as a result of mechanical properties involving foreign bodies. Abfraction is thought to be a type of fatigue wear, which occurs as a result of tensile or shear stress in the cemento-enamel junction and that initiate micro fractures in enamel and dentine (Lee and Eakle, 1984).

Tooth wear can lead to dentine exposure and DH and many clinical prevalence studies report DH in association with tooth wear (Fares *et al.*, 2009). Tooth wear leading to tooth loss have also been shown to cause social disabilities with speaking and chewing and psychological disabilities with appearance perception (Elias and Sheiham, 1998). It can impact on quality of life by causing pain, discomfort, less satisfaction in appearance and reduced eating capacity (Al-Omiri *et al.*, 2006).

1.5.1 Prevalence of tooth wear

The most common method used to measure the prevalence of tooth wear is to use the tooth wear indices. These are discussed in more detail in section 1.15.1 below. As shown, different researchers have explored this area in a variety of ways: with different indices, assessing different tooth surfaces, reporting on different age groups and presenting the data in a range of different ways. For example, they may report their finding at the subject level (reported as a percentage of subjects with tooth wear) or at the tooth level (reported as percentage of teeth with tooth wear). Variation in the data collected and analysis of the data makes comparison of studies difficult. Furthermore, many studies have small sample sizes and therefore care should be taken when relating their results to those of the general population (Nunn, 1996). Nonetheless, the conclusion from most studies is similar. Tooth wear, particularly erosive wear (Addy and Hunter, 2003; Dugmore and Rock, 2003; Nunn *et al.*, 2003), is a growing problem in adults (Van't Spijker *et al.*, 2009) and children (Nunn *et al.*, 2000); 4-5% of fifteen year-olds and 11% of adults in the UK have been shown to suffer from tooth wear (Chadwick and Penry, 2004; Nunn *et al.*, 2000) and this data suggests an increase over a ten year period (O'Brien, 1994). It has been shown that tooth wear has been almost universally experienced (Fares *et al.*, 2009).

1.5.1.1 Prevalence of tooth wear in children

The most comprehensive study of the prevalence of tooth wear in children was the 1993 United Kingdom Children's Dental Health Survey (O'Brien, 1993). This used a modification of the Smith and Knight Tooth Wear Index (Smith and Knight, 1984a) and reported on erosion in the upper incisors in 17, 000 children. Key findings from the study (O'Brien, 1993) included:

- Palatal erosion was more common that buccal erosion with 50% of six year olds showing erosion on the palatal surfaces and 19% showing erosion on the buccal surfaces,
- Erosion on the incisors was present in almost 25% of eleven year olds and 52% of 5-6 year olds,
- Erosion had progressed into the pulp in 25% of 5-6 year olds compared to 2% of 13 year olds,
- Erosion affecting permanent teeth was present in 31% of 14 year olds, with 32% having erosion on palatal tooth surfaces and 12% having erosion on buccal surfaces.

The 2003 Children's Dental Health survey showed that the proportion of 5 year olds with erosion on one or more buccal surfaces of primary upper incisors was 20% and 3% had erosion involving dentine or pulp. This is similar to the 1993 survey (18% and 1% respectively). Erosion of the lingual tooth surfaces was more common in the 2003 survey and affected 53% of 5 year olds. However, erosion into the dentine or pulp in 5 year olds was 22% and similar to the 1993 survey (25%). Erosion of the primary incisors was less than the permanent incisors. At age 8, 4% of incisors had erosion on the buccal surfaces and this had increased to 14% by age 15. Buccal erosion did not increase between 1993 and 2003 in the 8 year old age group, but there was a slight increase in buccal erosion in the 12 and 15 year old groups (Chadwick B and Penry, 2004).

1.5.1.2 Prevalence of tooth wear in adults

In a study of the prevalence of tooth wear on 93, 500 tooth surfaces in 1, 007 adults, it was shown that 98% had evidence of tooth wear (Smith and Robb, 1996). The tooth wear was recorded as un-acceptable and possibly pathological in 5.73% of tooth

surfaces in the 15- 26 year old age group, 8.19% of tooth surfaces in the 56- 65 year old age group and 8.84% of tooth surfaces in the over 65 year old group. For the three intermediate decades the prevalence was less and ranged between 3.37% and 4.62% (Smith and Robb, 1996). The level of wear classified as pathological was set in this study by the examiners who were experienced restorative specialists. The level of wear which is classified as un-acceptable is based on the current rates of tooth wear into the patient's life expectancy (Smith and Knight, 1984a). This explains why the level of tooth wear was recorded as less in the middle age groups, compared to the younger age groups. Likewise, the threshold for un-acceptable wear was increased in older age groups (Smith and Robb, 1996).

A systematic review of studies published between 1980 and 2007 showed that tooth wear increased with age (Van't Spijker *et al.*, 2009). The prevalence of adults with severe tooth wear increased from 3% at the age of 20 years to 17% at age 70. This is supported by the results from the recent 2009 Adult Dental Health Survey in the United Kingdom (Steele and O'Sullivan, 2009). The prevalence of tooth wear had increased from 66% in the 1998 Adult Dental Health Survey (Kelly *et al.* 1998) to 76% in the 2009 survey. In addition, moderate tooth wear had increased from 11% in the 1998 survey to 15% in 2009, although severe tooth wear remained rare (2%). The greatest increase was in the youngest age groups, between 16 and 44 years old, where wear and in particular moderate wear increased. The prevalence of tooth wear in anterior teeth was high (77%). There were also geographical variations in wear.

1.5.1.3 Incidence of tooth wear

Compared to prevalence studies, which show the occurrence of tooth wear at a specific time point, incidence studies show the appearance of new disease in a population over

a time period and represent disease development. One study examined tooth wear in orthodontic study casts taken over a five-year period and showed that the incidence of erosive lesions of 18% in children aged 10-15 years (Ganss *et al.*, 2001). Another study of 1, 308 adolescents showed that 12.3% of the population developed erosive lesions over a period of 2 years. Another study on 500 sets of study casts taken over an 18 month period showed that the tooth wear process is slowly progressive (Bartlett, 2003). This study however was not correlated to risk factors. In addition, the sample sizes of studies to date, which investigated the rates of tooth wear and correlated this to risk factors, are small.

1.6 Tooth wear and DH

Physiological tooth wear allows time for the pulp to lay down reparative or secondary dentine, which could prevent fluid flow within dentine tubules and also reduce DH (Krauser, 1986a). In contrast, during pathological tooth wear, the symptoms of DH are reported more frequently (Absi *et al.*, 1987; Addy and Pearce, 1994; Addy, 2000; 2002; Dababneh *et al.*, 1999; Smith and Knight, 1984b). DH could therefore be an important clinical indication of a pathological wear process (Dababneh *et al.*, 1999), which if undisturbed could lead to an increase in the frequency and severity of tooth wear lesions (Addy, 2002).

As pathological tooth wear lesions progress further into dentine, the radius of dentine tubules becomes larger and the distance to the pulp decreases. As a result, the hydraulic conductance of fluid within dentine (and DH symptoms) would be expected to increase (Pashley, 1990c). However, exposure of dentine will not necessarily lead to the presence of DH *per se* (Absi *et al.*, 1987; Yoshiyama *et al.*, 1996). Hence for the purposes of understanding the aetiological processes involved in DH, the names

'lesion localisation' and 'lesion initiation' were proposed (Addy, 2002). Lesion localisation involves dentine exposure, which may occur as a result of enamel or dentine wear or gingival recession. Lesion initiation may arise following lesion localisation and involves exposure of patent or un-occluded dentine tubules from the surface of dentine to the pulp. This often occurs following loss of the smear layer, which is a thin 'loose' layer consisting of organic collagen and glycosaminoglycans that forms an adherent matrix for mineralised tissue arising from saliva and dentine particles that might occlude the dentine tubules (Brannstrom, 1966; Pashley, 1984). Clinical studies lend support to lesion initiation and demonstrate why dentine exposure will not necessarily lead to DH if the dentine tubule system is not patent. In two *in vivo* studies, cavities were prepared in dentine and hydrostatic pressures were applied to the exposed dentine. Patients reported sensations of short sharp pain in those lesions in which the smear layer was removed from the surface of the prepared cavity using an acid challenge, but not in lesions in which the smear layer was present (Ahlquist *et al.*, 1994; Brannstrom, 1965). Clinical observation studies also show that DH can be uncommon even in cases where the pulp is visible through a thin bridge of dentine (Bartlett and Ide, 1999), which is likely to consist of sclerotic or transparent dentine (see section 1.2). Therefore, it should be noted that in addition to the smear layer or other surface occlusion, the degree of sclerosis by peritubular dentine and the extent of occlusion by reparative dentine on the pulpal surface might also affect the capacity for fluid movement within dentine tubules (Yoshiyama *et al.*, 1996).

1.7 Erosion

Erosion is now considered to be the most common and important aetiological factor for tooth wear in Europe (Addy and Hunter, 2003; Deery *et al.*, 2000; Grippo *et al.*, 2004; Lussi *et al.*, 2006; Seligman *et al.*, 1988). It arises due to superficial demineralisation of

hard tissue and chemical dissolution of apatite crystals by an acid (Bartlett, 2005a) that is not produced by the oral flora, but from intrinsic or extrinsic sources (Ten Cate *et al.*, 2008).

Typical sources of intrinsic acid are regurgitated stomach acid containing hydrochloric acid (HCL) due to vomiting or gastro-oesophageal reflux (Scheutzel, 1996). Typical extrinsic sources are given below (Lussi, 2006; Zero, 1996);

- Diet (for example, acidic citrus and other fruits, carbonated beverages and sports drinks, beers and herbal teas, vinegars and pickles, candies),
- Medicaments (for example non encapsulated HCL replacement, chewing ascorbic acid tablets (vitamin C) and acetylsalicylic acid tablets (aspirin), iron tablets, salivary stimulants,
- Occupation (for example jobs involving wine tasting or working near acidic industrial vapours),
- Sports (for example improperly chlorinated swimming pools).

Enamel and dentine vary in composition as well as morphology and therefore the processes involved in erosion of enamel and dentine are quite different. Enamel consists of 96% inorganic matter and 0.1% organic matter by dry weight. Dentine consists of 72% inorganic by dry weight, 18% collagen and 2% other organic material (Williams and Elliott, 1989). In enamel, hydroxyapatite reacts with the acid hydrogen (H^+) ion resulting in the formation of calcium citrate followed by the chelating (calcium binding) action of calcium citrate. It was shown *in vitro* that immersion of human enamel samples in acid resulted in erosion first in the prism sheath area of the enamel followed by the prism cores and then the inter-prismatic areas over time, as observed using SEM (Meurman and Frank, 1991). In dentine, it was observed that the acid first

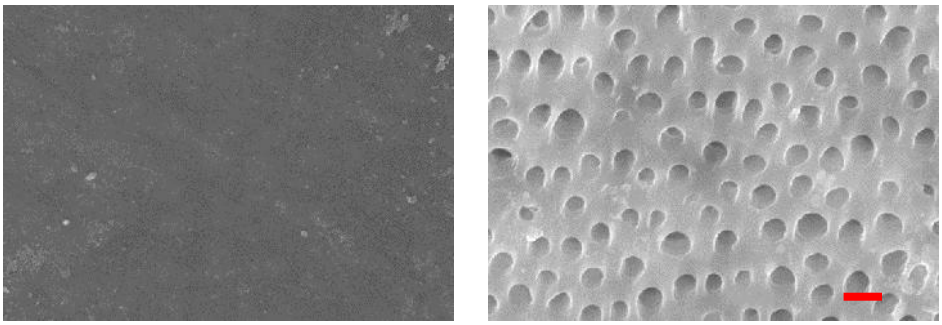
affects the interface between the peritubular and inter-tubular dentine with dissolution of the peritubular dentine followed by the inter-tubular areas (Meurman *et al.*, 1991).

1.7.1 Erosion and DH

It was demonstrated that a 6% solution of citric acid would remove the smear layer from dentine in successive layers based on etching time (Pashley *et al.*, 1981). Then, in an *in vitro* study by Addy *et al,* the effects of acids and dietary substances on root planned and burred dentine were investigated (Addy *et al.*, 1987a). The effects of the acid were to remove the smear layer and expose patent or un-occluded dentine tubules. It was concluded that the presence of these patent dentine tubules is related clinically to DH.

Table 1 shows SEM images of the surface of root taken from the buccal cervical region of a premolar tooth before and after an acid challenge using 6% citric acid. Dentine tubules are visible throughout the surface of the root dentine that has been exposed to an acid challenge in contrast to the root dentine left unchallenged. This is because the smear layer has been lost due to the acid challenge. Most of the dentine tubules are greater than 1µm diameter post-acid challenge. This is greater than 0.83µm, the minimum diameter reported as being required to elicit DH at the cervical area of the tooth near the DEJ (Absi *et al.*, 1987).

Table 1 Scanning electron micrograph (SEM) images (x2000) of untreated root surface (left) and root surface following a 1 minute 6% citric acid challenge with gentle agitation (right). Scale bar 2μm.



There is an increasing body of literature indicating that acid erosion caused by relatively small acidic challenges will lead to loss of enamel and dentine and expose the dentine tubules. This literature includes laboratory research (Absi *et al.*, 1992; Addy *et al.*, 1987a; Ganss *et al.*, 2009; Gregg *et al.*, 2004; Vanuspong *et al.*, 2002; West *et al.*, 1999), review papers (Addy, 2005; Lussi, 2006; Zero and Lussi, 2005), clinical research (Absi *et al.*, 1992; Hughes *et al.*, 1999; Hunter *et al.*, 2000) and prevalence studies (Lussi and Schaffner, 2000; Smith *et al.*, 2008). These studies mention the importance of reducing the erosive potential of many popular acidic beverages.

1.7.2 Erosive potential

An acid is a hydrogen ion donor and a base is a hydrogen ion recipient (Williams and Elliott, 1989). The severity of erosive challenge caused by an acid is likely to decrease with pH and increase with titratability, time of acid challenge, temperature and ion concentration, frequency of acid challenge and presence of chelating agents (Moss, 1998; West *et al.*, 2000). There are a number of foodstuffs that contain acids and are popular consumables in the UK. These are summarised in Table 2.

Table 2 Erosive foodstuffs and their acids

Beverage	Acid
Citric fruits including oranges, lemons, grapefruit	Citric acid
Apples, plums and peaches	Malic acid
Grapes and wines	Tartaric acid
Fermented products and yogurt	Lactic acid
Preservative	Acetic acid
Rhubarb	Oxalic acid
Cola drink	Phosphoric acid

The pH is a popular reported measure of acidity. It describes the dissociation of acids hydrogen ions in water such that acids with a lower pH have more dissociation of hydrogen (H^+) ions in water and are therefore stronger acids (Williams and Elliott, 1989). Described more eloquently, pH provides a measure of the concentration of hydrogen ions in solution and the acids pKa (or acid dissociation constant) measures how much of an acid can dissolve in solution. Carbonated drinks such as Coca-Cola and lemonade both contain carbon dioxide under pressure, giving a solution of carbonic acid, which has been shown to have considerably lower pKa and pH values than citric and malic acids. Although acids with lower pH and pKa would be expected to create more erosive wear and exposure of dentine tubules, this has been refuted in the literature. For example, an early *in vitro* study, which investigated the effect of various dietary beverages on dentine using SEM, showed that many popular erosive beverages of low pH would not lead to exposure of patent dentine tubules (Addy *et al.*, 1987a). These included a low pH carbonated drink, Coca-Cola, and Ribena (a fruit based soft drink). Instead, consumables such as red and white wine, citrus fruit juices, apple juice and yogurt did produce visible dentine tubules using SEM (Addy *et al.*,

1987a). Citric acid was also described at the time as most detrimental to human enamel (Meurman *et al.*, 1987).

For the purposes of investigating the dental erosive potential of acids used during the work by Addy (Addy *et al.*, 1987a), a laboratory study was conducted by Grenby and demonstrated that titratability is likely to be more important than pH in determining erosive potential (Grenby *et al.*, 1989). For example, the Coca-Cola drink followed by Ribena, were shown to have substantially lower titratable acidities in contrast to fruit juices (lemon, orange and pineapple). Titratable acidity (or neutralisable acidity) is the volume of alkali required (typically 0.1mol solution of sodium hydroxide) to raise the pH of a standardised volume of beverage (typically 25ml) to pH 7 (Chadwick, 2006). In dental erosion, titratability provides an indication of the actual concentration of hydrogen ions available to interact with a mineralised surface, which provides an indication of the erosive potential (Zero, 1996).

Another important aspect to acids found within popular erosive beverages are that they are not simple chemical solutions, but might in addition contain a variety of components such as particulate matter associated with high calcium (Ca) and phosphate (P) levels, which could assist in a buffering action and potential to maintain a gradual release of acid dissolving the tooth mineral (Grenby *et al.*, 1989). Fruit juices, such as orange or pineapple juice result in more demineralisation than other popular erosive beverages such as the Coca-Cola and Lemonade drinks, which have lower levels of Ca and P (Grenby *et al.*, 1989). This is dependent on the calcium binding property, otherwise known as chelation of an acid, which is a complex between the calcium cation and two or more groups producing a ring structure that includes water. Acids such as citric, malic and tartaric acid contain more than one carboxyl group in their chemical composition and this result in them being able to bind more than one calcium ion at

high pH (Meurman *et al.*, 1987). Calcium binding from saliva will result in loss of the ion effect of calcium in saliva, which leads to more dissolution tendency. Also, if the calcium in saliva is bound, there may be more tendencies for dissolution of dental tissue to replace that lost in saliva (Meurman and tenCate, 1996). This results in more erosion.

More recent *in vitro* metrological investigation of erosion using human dentine (taken from the cervical area of teeth) examined the depth of erosive lesions when immersed in various concentrations of acid followed by ultrasonication to remove the softened dentine layer (Vanuspong *et al.*, 2002). The depths of erosive lesions were shown to increase significantly with time and decrease with higher pH. Another *in vitro* study using bovine dentine samples showed that dental wear is greater following acid challenges of longer duration, increasing flow rates, greater titratable acidity (such as citric compared to hydrochloric acid) and lower pH (Wiegand *et al.*, 2007). This study also used human saliva. Review papers highlight the importance of a mature salivary pellicle in protecting against erosion; in particular that the phosphate, calcium and fluoride content of an erosive challenge may prevent dental wear (Zero and Lussi, 2005). This also emphasises the importance of salivary factors in affording a protective role during the tooth wear process and the importance of saliva in research studies on erosion.

As mentioned, the frequency and quantity of acid challenge will affect erosion. However, in 1997 it was reported that UK soft drinks consumption had risen by 56% in the previous 10 years and was predicted to continue rising 2-3% each year thereafter (Zenith-International-Ltd, 1997). This predicted increase is confirmed according to a recent report from the British Soft Drinks association (BSDA, 2011). Table 3 shows UK soft drink consumption between 2004 and 2010. Consumption of all beverages, except

bottled water, has increased. Of particular concern are consumed acidic beverages, such as juice drinks and fruit juices, which have been shown to cause dental wear and exposure of dentine tubules and for which consumption has increased significantly between 2004 and 2010 (BSDA, 2011).

Table 3 UK soft drink consumption between 2004 and 2010

UK soft drinks	Million litres consumed in the UK	
	2004	2010
Carbonates	6195	6400
Dilatable drinks	3125	3500
Bottled water	2060	2055
Still and juice drinks	1090	1450
Fruit juice	1040	1180
Sports and energy drinks	320	600

The importance of popular erosive beverages in erosion and DH and their increased consumption is of particular concern. Investigation of these aetiologies in tooth wear and DH is required clinically.

1.8 Abrasion and DH

Abrasion is a physical process, which occurs as a result of the mechanical wear of dental tissues by foreign bodies. Tooth brushing and dentifrices are common forms of dental abrasion (Addy and Hunter, 2003). Toothbrush abrasions are influenced by brushing habits, force applied and the time spent brushing (Hooper *et al.*, 2003). There are additional habits linked to abrasion, such as onychophagia, clips and other tools, which may come into contact with teeth. Unlike erosion, less evidence exists to support

the importance of tooth brushing and dentifrices in causing DH (Abrahamsen, 2005; Addy and Hunter, 2003; Bartlett and Shah, 2006; Ganss *et al.*, 2009).

1.8.1 Tooth brush abrasion

The effects of normal tooth brushing on wear of enamel are negligible and unlikely to lead to exposure of the underlying dentine alone unless erosion is also occurring (Addy, 2005). It was shown *in vitro* that increasing water temperature does not have an effect on dentine wear with normal tooth brushing (Scaramucci *et al.*, 2009). However, when the force applied to dentine *in vitro* is increased from 90 g to 100 g and if manual toothbrushes are used instead of powered toothbrushes, it has been shown to cause more dentine wear (Knezevic *et al.*, 2010). It is suggested that the force applied to dentine with a manual brush is higher than a powered brush and therefore manual brushes can overtime cause more wear. This claim is also supported by a recent systematic review of the literature including evidence from prevalence studies (Van der Weijden *et al.*, 2011).

The filament stiffness of toothbrushes is also important in dentine wear. Smaller filament stiffness (decreasing diameter of filament) has been shown *in vitro* to cause higher wear in dentine using various abrasivities of dentifrice (post acid erosion), but the effect of stiffness on dentine wear is less than the dentifrice abrasivity (Wiegand *et al.*, 2009). Prevalence studies show that there is a significant correlation between patients who brush using a medium and hard, rather than a soft, stiffness toothbrush and NCCL's (Smith *et al.*, 2008).

1.8.2 Tooth brush abrasion and DH

It has been shown that teeth with lower plaque scores (such as left hand side teeth in patients who are right handed) are more likely to have DH and research supports the association of buccal tooth brushing habits with gingival recession and DH (Addy *et al.*, 1987b; Addy *et al.*, 1987d). A laboratory study investigating dentine wear has shown that the effect of normal tooth brushing alone even for extended time periods measured in years will cause limited wear on the dentine itself and that this wear may be limited to the smear layer (Absi *et al.*, 1992). Prevalence studies suggest that DH is more commonly reported on buccal tooth surfaces and often in association with NCCL's, as mentioned in section 1.4. Prevalence and clinical studies show that the presence of NCCL's and DH may be linked to inappropriate brushing techniques (too hard or frequent), which leads to dentine exposure as a result of gingival recession (Addy and Hunter, 2003; Chabanski *et al.*, 1996; Hooper *et al.*, 2003; McCracken *et al.*, 2003; Smith *et al.*, 2008). One clinical study showed that tooth brushing with more than 100 g force is linked to gingival recession using a powered toothbrush (McCracken *et al.*, 2003). It is reported that when greater forces are applied to healthy teeth using a manual toothbrush, patients are more likely to report pain that resembles DH (Addy, 2005). These studies suggest a link between abnormal oral hygiene procedures and gingival recession or tooth wear and, in turn, DH. This is reported in the literature (Addy and Hunter, 2003). Further clinical work is necessary to confirm an association between tooth brushing habits, tooth wear and DH.

1.8.3 Dentifrice abrasion

Dentifrices or toothpastes are routinely used by patients owing to their therapeutic and cosmetic purposes, but due to abrasivity are likely to cause more wear of enamel and

dentine than normal tooth brushing alone (Wulknitz, 1997). Nonetheless, tooth brushing with dentifrices in the absence of acid is likely to cause little or no wear of enamel because the abrasives contained with the dentifrice, with the exception of non-hydrated alumina, are softer than enamel (Addy, 2005). In dentine, a review of the literature suggests that a cumulative abrasion of 1mm would take place during 80-100 years of tooth brushing with toothpaste (Hunter *et al.*, 2002).

During abnormal or abusive use, tooth brushing with toothpaste can lead to pathological wear as discussed in a review of the literature (Hunter *et al.*, 2002). This has been supported by *in vitro* studies, which show that increasing concentrations of dentifrice mixed with artificial saliva or higher dentifrice abrasivity are likely to result in more dentine wear using a 300g constant force (Hooper *et al.*, 2003; Turssi *et al.*, 2010). Dentifrice abrasivity is measured using the RDA (Relative Dentine Abrasivity), which is a numeric value. It is calculated based on *in vitro* methods that investigate the ability of dentifrice slurry to remove radioactive enamel or dentine during a brushing protocol relative to standard abrasive or dentifrice. The rate of human dental wear has been shown *in situ* to correlate reasonably well with RDA (Addy *et al.*, 2002). Another study reported mean dentine wear was in the range 0.28-27.63μm and that dentine wear increased significantly ($p<0.05$) as dentifrice concentration increased from an RDA of 90 to 200 (Hefferen, 1976; Macdonald *et al.*, 2010). However, another *in situ* study using commercial dentifrices showed that variations in RDA value in between 90 and 352 caused no statistically significant difference in the amount of tooth wear over 12 and 24 weeks of brushing (Pickles *et al.*, 2005). Differences in wear were only noticed between these commercial dentifrices and an experimental paste with an RDA value of 4. The research might suggest that whilst *in vitro* tests have value in predicting differences in RDA between products on tooth wear; they may not always be ideal to predict the effects that might occur clinically.

The International Standards Organisation states that for dentine, the abrasivity of test formulation should not exceed 2.5 times the reference abrasive i.e. RDA must not exceed 250 (International.Standards.Organisation.ISO.11609, 1995). All dentifrices marketed for relief of DH have RDA values below 100. Interestingly, the allowed pH range for dentifrice (pH 4-10) might be more cause for concern as it suggests that some dentifrices of low pH could intrinsically lead to chemico-physical dental wear. Despite this, ISO standards ensure all products are above a pH that may cause demineralisation (pH 5.5 for enamel and pH 6.5 for dentine) or the contained fluoride balances the low pH effect (Hunter *et al.*, 2002). Furthermore, following an erosive acid challenge, dental tissue in solution supersaturated with respect to tooth tissue has been shown *in vitro* to not dissolve and the effect is enhanced with the use of fluoride. Therefore, dentifrices with a higher buffering capacity that actively encourage mineral uptake create less tooth wear abrasion than those with lower buffering capacities (Betke *et al.*, 2003; Zero and Lussi, 2005). Unfortunately, many of the studies investigating the influence of dentifrices on abrasion and DH are based on *in vitro* studies or anecdotal reports, with few *in situ* and no *in vivo* studies (Addy, 2005).

1.8.4 Dentifrice abrasion and DH

Although dentifrices may cause limited tooth surface loss, it is reported that dentifrices of greater abrasivity will cause DH lesion initiation by removal of the smear layer and establishment of patent dentine tubules (Addy and Hunter, 2003). Despite this, it has been shown *in vitro* that some dentifrices, especially those containing silica, may slightly occlude the dentine tubules (Addy and Mostafa, 1989; West *et al.*, 2002). Another *in situ* study showed that tooth brushing, in combination with dentifrices, could

even provide a therapeutic action, in creation of a smear layer and prevention of DH (Addy *et al.*, 2002).

1.9 Attrition and DH

Attrition is the physical wear of dental hard tissues due to tooth to tooth contact. In normal function, the teeth only contact for a short period of time for eating or swallowing. However, when this contact occurs at other times, it is termed parafunction or bruxism. This often occurs nocturnally as a form of stress relief (Bartlett and Smith, 2000). Prevalence studies show that the occlusal as well as buccal tooth surfaces might demonstrate DH, although DH is more common on buccal tooth surfaces in association with gingival recession (Bamise *et al.*, 2008).

1.10 Abfraction and DH

Tooth wear lesions that cannot be explained due to erosion and or abrasion and which occur due to occlusal stress often occur near to the cervical margin of teeth (Bevenius *et al.*, 1993) and were later described by Grippo as abfraction lesions. These were proposed in 1991 as the primary cause of NCCL's (Grippo, 1991b) and NCCL's have been associated with DH (Addy, 2002). Furthermore, Lee and Eakle, in a position paper, suggested that abfraction is as important as erosion and abrasion in the aetiology of NCCL's (Lee and Eakle, 1984). Despite some correlation between occlusal stress and non carious cervical wear (Smith *et al.*, 2008), critical reviews of the literature provide little evidence linking occlusal stress with cervical wear and therefore the important aetiologies appear to be other factors, such as erosion and abrasion (Bartlett and Shah, 2006).

1.11 Periodontal disease and DH

Periodontal disease is also linked to DH indirectly through gingival recession (Chabanski *et al.*, 1996; Madhu and Setty, 2006) and tobacco is a significant aetiology of periodontal disease throughout Europe (Olley and Gallagher, 2010). Therefore, oral hygiene, periodontal status and tobacco use are all relevant aetiologies in DH. Patients who have DH may be less likely to brush their teeth due to pain. This increases the level of plaque on their dentine, which reduces DH, but increases their likelihood of periodontal disease.

1.12 Multi-factorial aetiologies of tooth wear

In tooth wear, it is unusual that attrition, abrasion or erosion should occur individually and it may be more accurate to describe them; as in a previous review; through dental tribology as two body, three body and chemico-physical wear respectively (Addy, 2005). For patients, these wear processes may include oral hygiene practices, dietary habits, stress and its effects on the occlusion (Bartlett and Shah, 2006; Bartold, 2006; Shah *et al.*, 2009). Dental erosion often works in synergy with abrasion, in the aetiology of NCCL's and DH (Lussi, 2006) and tooth brushing will at the very least remove the acquired pellicle, which has been shown to offer protection against erosion *in vitro* (Wetton *et al.*, 2006).

It has been shown in laboratory studies that dentifrices of higher RDA value may cause more dentine wear, but it should also be noted that in some of these studies (Hooper *et al.*, 2003; Wiegand *et al.*, 2009) the wear was measured post-acid challenge (using hydrochloric acid). Recent laboratory research suggests the importance of erosion may make abrasion insignificant. One *in vitro* study investigated the effect of tooth brushing

forces up to 400g on dentine samples, which had been eroded using HCL for 6 x 2 min d^{-1} in a cyclic demineralisation and remineralisation protocol (Ganss *et al.*, 2009). Interestingly, it found that dentine wear and mineral loss were greater in dentine samples that had been eroded only compared to those which had been eroded and then abraded by an electric toothbrush and dentifrice, regardless of force applied. The authors suggested this maybe because the remaining demineralised dentine has excellent tensile properties. A previous study of dentine reported values of 30 MPa for ultimate tensile strength and 0.25 GPa for modulus of elasticity (Sano *et al.*, 1994). It should be noted that this value is slightly higher than average and may be due to the static versus dynamic methods, where dynamic testing will produce a range of modulus of values (Rees *et al.,* 1994). Nonetheless, Ganss *et al.* 2009 suggested that the collagen matrix may be resistant to tooth brushing and could remineralise. The results of this research suggest that the role of erosion is far important than abrasion in tooth wear. For this purpose, it might raise questions over the current recommendation by the dental profession to avoid brushing (dentine) immediately after consuming acidic food or drinks (Dababneh *et al.*, 1999), albeit the relation of these findings to the clinical situation is unknown.

1.13 Multi-factorial aetiologies of DH

Combinations of tooth brushing with an erosive acid challenge will enhance removal of the smear layer and it was suggested in an early *in vitro* study that brushing should be avoided immediately after meals (Absi *et al.*, 1987). Another *in vitro* study showed that tooth brushing alone may take several years to remove the smear layer and expose the dentine tubule system, but this exposure occurs more readily if followed by an erosive acid challenge (Absi *et al.*, 1992). Clinically, it has also been shown that tooth brushing immediately post acid challenge can lead to more DH like symptoms (Addy, 2005;

Ahlquist *et al.*, 1994). Evidence therefore supports the role of multiple factors, in particular erosion and abrasion, in the aetiology of DH, even though one factor may be dominant. The synergy between the various aetiologies is partially reflected in the current clinical suggestion from the dental profession to avoid tooth brushing immediately after acidic food or drink consumption (Dababneh *et al.*, 1999).

Over the past twenty years, the predominant aetiologies involved in NCCL's and DH in the literature have changed. In 1984, a case study and review reported that these were most likely to be due to abfraction, erosion and abrasion (Lee and Eakle, 1984). Then in 1996 a prevalence study on 1,007 dental hospital patients attributed the main aetiologies as erosion and abrasion (Smith and Robb, 1996). More recently, erosion was described as the predominant aetiology (Addy and Hunter, 2003). Overall, the research suggests that erosion is important in exposure of dentine tubules and initiation of a DH lesion. Abrasion alone may have an aetiological or even therapeutic role in combination with specific dentifrices as described in section 1.8.4. Studies still support a role for multi-factorial aetiological factors in DH, but extrapolation of these findings to the clinical setting is unknown.

1.14 Management of DH

A large array of treatments is available for DH, but none are definitive perhaps due, in part, to a historical lack of understanding of the risk factors involved in DH, the effect of treatment agents applied to dentine and validity of management strategies. Indeed, DH was first described as an enigma thirty years ago and this concept has been re-visited recently and on several occasions in the intervening period (Addy, 2002; Dababneh *et al.*, 1999; Johnson *et al.*, 1982; Markowitz and Pashley, 2008). This has resulted in treatment and preventive strategies that have often been elusive and which focus on

the symptoms of DH rather than its cause/s (Markowitz and Pashley, 2008). It was mentioned earlier that dietary acids are one of the most important currently accepted aetiology in DH (Addy, 2005). It is nonetheless important to first establish a clear diagnosis of DH, identify its causative factors and ensure their avoidance to help inform a successful management strategy (Dababneh *et al.*, 1999). Then, management strategies should focus on the managing the aetiology of DH and not just its symptoms. Secondly, treatments include agents in dentifrices or mouth rinses for home use or professionally applied varnishes. These are extremely popular in the general population and are available over the counter. Thirdly, restorative treatment may be indicated as a last resort.

1.14.1 Mechanism of action of desensitising dentifrices

Dentifrices are routinely used for the treatment of DH. As well as the active components used for their anti-caries, desensitising or anti-microbial properties, dentifrices also contain excipients, summarised in

Table 4, adapted from (Davies *et al.*, 2010; Kidd, 2005; Scheie and Peterson, 2008).

Table 4 Excipients used in dentifrices

Ingredient	**Purpose**
Abrasives for cleaning, polishing and stain removal (30-40%)	Hydrated alumina, Aluminium trihydrate, Bentonite, Calcium carbonate, Calcium pyrophosphate, Dicalcium phosphate, Kaolin, Methacrylate Perlite (a natural volcanic glass), Polyethylene, Pumice, Silica, Sodium bicarbonate, Sodium metaphosphate.

Ingredient	Purpose
Surfactants for foam and detergent action (1-2%)	Amine fluorides, Dioctyl sodium sulfosuccinate, Sodium lauryl sulfate (SLS), Sodium N lauryl sarcosinate, Sodium stearyl fumarate, Sodium stearyl lactate, Sodium lauryl sulfoacetate.
Humectants (10-30%)	Xylitol, Glycerol, PEG 8 (polyoxyethylene glycol esters), Pentatol, PPG (polypropylene glycol ethers), Sorbitol, Water.
Gelling or binding agents for rheology and to carry the abrasive and active ingredients (1-5%)	Carbopols, Carboxymethyl cellulose, Carrageenan, Hydroxyethyl cellulose, Plant extracts (alginate, guar gum, gum arabic), Silica thickeners, Sodium alginate, Sodium aluminum silicates, Viscarine, Xanthan gum.
Flavouring agents to make tooth brushing taste more pleasant (1-5%)	Aniseed, Clove oil, Eucalyptus, Fennel, Menthol, Peppermint, Spearmint, Vanilla, Wintergreen.
Preservatives (0.05-0.5%)	Alcohols, Benzoic acid, Ethyl parabens, Formaldehyde, Methylparabens, Phenolics (methyl, ethy, propyl), Polyaminopropyl biguanide.
Colouring agents	Chlorophyll, Titanium dioxide.
Film agents to give a smooth, moist feel	Cyclomethicone, Dimethicone, Polydimethylsiloxane Siliglycol.
Sweeteners	Acesulfame, Aspartame, Saccharine, Sorbitol.

Active ingredients designed to desensitise the tooth have one or two modes of action (Krauser, 1986b);

1. Nerve depolarisation,

2. Tubule occlusion.

For many years, attention focused on dentifrices containing ingredients, which 'desensitised' the dental nerves and therefore managed the symptoms of DH. These agents included various potassium salts such as potassium nitrate, potassium chloride and potassium citrate (Schiff *et al.*, 1994). Numerous clinical trials have been conducted *in vivo* on nerve depolarisation agents (such as potassium ions) (Schiff *et al.*, 2000). In a recent systematic review of potassium containing dentifrices used to treat DH (Poulsen *et al.*, 2006), six studies in the meta-analysis identified a statistically significant effect of potassium nitrate dentifrice on stimuli, which included tactile and air blast, at the six to eight week follow up. However, it was concluded that the evidence for potassium salts in the management of DH is unclear, due to variation in the methods applied for assessing sensitivity and the small sample numbers in a variety of studies (Poulsen *et al.*, 2006). It is also not easy to investigate the uptake of desensitising agents and quantify this and is complicated by the outward flow of dentinal fluid (Gillam D.G. *et al.*, 2000). Furthermore, unlike tubule occluding agents, nerve depolarisation ingredients do not physically block the entry to dentine tubules and therefore would not be expected to protect the dentine against exposure of additional dentine tubules following aetiologies such as an acid challenge (Banfield and Addy, 2004; Markowitz and Pashley, 2008).

Ingredients specifically used in dentifrices to occlude dentine tubules have included arginine, calcium hydroxide, calcium phosphate, cyanoacrylate, dicalcium phosphate, ferric oxalate, formalin, glycerin, potassium nitrate, potassium oxalate, resins, silica, strontium acetate, strontium chloride, silver nitrate, sodium citrate, sodium fluoride and stannous fluoride (Addy and Mostafa, 1989; Ling and Gillam, 1996; Suge *et al.*, 2005). Some of these have poor tubule occluding properties or are acid labile, which is

problematic considering erosion is one such aetiology of DH. For example, products including the oxalates and calcium phosphates have been shown not to occlude the dentine tubules *in situ* to following an acid challenge using the erosive beverage orange juice (Banfield and Addy, 2004). Calcium sodium phosphosilicate (contained in NovaMin®), Arginine and stannous fluoride are relatively recent additions to the dentine tubular occlusion technologies (Garcia-Godoy, 2009; Gillam *et al.*, 2002), whereas strontium has been used in dentifrices for over half a century (Kanapka, 1990). Clinical studies and laboratory research has been conducted to investigate the desensitising effects of tubular occluding agents; arginine, calcium sodium phosphosilicate, strontium and stannous fluoride hexametaphosphate. Recent commercially available dentifrices based on similar or new occlusion technology and which have demonstrated some acid resistant properties include:

- Colgate Sensitive Pro-Relief® Daily Paste which contains a Pro-Argin™ formulae (containing 8% Arginine and Calcium Carbonate as well as 1450ppm Sodium monofluorophosphate),
- Sensodyne® Rapid Relief (containing 8% Strontium Acetate and 1040ppm Sodium fluoride),
- Sensodyne® Repair and protect with 5% NovaMin® (containing a calcium sodium phosphosilicate bioglass),

One clinical study showed that a 8% arginine-based dentifrice reduced DH immediately (Schiff *et al.*, 2009) following a three day (Fu *et al.*, 2010) and eight week (Que *et al.*, 2010a) application period compared to controls using 2% potassium nitrate or citrate and 1450ppm sodium monofluorophosphate fluoride based dentifrices. Although 8% arginine-based dentifrices also demonstrate dentine tubular occlusion and resistance to an acid challenge in one *in vitro* study (using Coca-Cola drink) (Lavender *et al.*, 2010)

other *in vitro* studies (using grapefruit juice) refute the latter (Parkinson *et al.*, 2010; Sauro *et al.*, 2010).

An early *in situ* randomised control trial using dentine samples treated with strontium in silica compared to control, showed a dentine surface deposit resistant to water rinsing under SEM (Addy *et al.*, 1987c; Addy and Mostafa, 1989). More recently, laboratory work has shown that a strontium-based dentifrice may also have acid resistant properties. Studies conducted *in vitro* show that 8% strontium-based dentifrices produce significant dentine tubular occlusion following acidic challenge (using grapefruit juice) (Banfield and Addy, 2004; Claydon *et al.*, 2009; Parkinson *et al.*, 2010). Similarly, an 8% strontium-based dentifrice in silica base reduced DH significantly compared to a 1450ppm control paste (sodium fluoride in silica base) (Mason *et al.*, 2010) and an 8% arginine and 1450ppm sodium monofluorophosphate (Hughes *et al.*, 2010).

More recent agents used in dentine tubule occlusion include calcium sodium phosphosilicate (NovaMin®). A 5% calcium sodium phosphosilicate was shown to reduce DH compared to 5% potassium nitrate in a clinical trial (Pradeep and Sharma, 2010). Laboratory studies show that compared to controls, dentine samples treated with a 5% calcium sodium phosphosilicate have fewer patent tubules, greater surface hardness and the release of calcium over time is claimed to provide continual occlusion of dentine (Burwell *et al.*, 2010). Bioactive glass particles have also been shown to produce dentine tubule occlusion *in vitro* (Gillam *et al.*, 2002).

Finally, laboratory research shows stannous fluoride based dentifrice have been shown to cause dentine tubule occlusion *in vitro* and following a 3 minute acid challenge (using the drink Coca-Cola) (Von Koppenfels *et al.*, 2005). It also shows that stannous

fluoride hexametaphosphate dentifrice provides an acid resistant layer to dentine tubule exposure and prevents dental demineralisation post acid softening (White *et al.*, 2007; Zsiska *et al.*, 2010). Stannous fluoride based dentifrices have also been shown to reduce DH at 4 and 8 weeks in subjects who are suffering from DH (Schiff *et al.*, 2006).

Another recent *in vitro* study using dentine samples treated with dentifrice containing polymethyl vinyl ether-maleic acid in a silica base demonstrates occlusion of dentine tubules and resistance to a 10 minute orange juice challenge compared to dentifrice without this copolymer (Liu *et al.*, 2011).

1.15 Measurement of DH and tooth wear

There has been a lack of research on the aetiology, effect of treatments and management strategies involved in DH. Most of the research on DH has been conducted *in vitro,* with some *in situ* and few *in vivo* studies (Addy, 2002; Markowitz and Pashley, 2008). DH has also been described as a tooth wear phenomenon (see section 1.6) and therefore studies that investigate DH might rely on accurate measurement of tooth wear as well as DH. However, tools to measure tooth wear in terms of surface loss alone may not reflect the presence of DH because, as stated previously, exposed dentine may not have DH symptoms (Absi *et al.*, 1987; Addy, 2002; Yoshiyama *et al.*, 1996). Tooth wear was described in relation to DH earlier as lesion localisation (which will not necessarily involve symptoms of DH) and lesion initiation (which is likely to involve the presence of patent or un-occluded dentine tubules and symptoms of DH) (Addy, 2005). Therefore in addition to measuring tooth wear, it is important to measure DH for the purpose of diagnosis and, in the case of research on management strategies, to address the efficacy of various desensitising

treatments. DH may be measured directly (from the presence or absence of clinical symptoms) or indirectly (in the laboratory by investigating the presence/absence/size of dentine tubules).

To date, epidemiological studies have been conducted to measure DH or tooth wear using a variety of techniques. Clinical studies using subject reported pain levels may be used to observe DH or to investigate treatments in particular desensitising dentifrices. In addition, laboratory or *in situ* studies have been conducted using replica impressions taken of the dentine surface or directly on dentine samples or recently extracted teeth. These samples or impressions have then been imaged or investigated for DH and or tooth wear using various metrological devices. Subsequently, techniques have been devised to measure the amount of dentine tubule occlusion from images. Studies *in vivo* to investigate dentine tubules following various aetiologies and treatments are difficult to perform. Studies therefore rely on *in situ* studies using some techniques *ex vivo* or carried out in the laboratory.

The main measurements relevant to this PhD include clinical indices used to measure tooth wear and DH, and imaging techniques including Scanning Electron Microscopy (SEM) and Tandem Scanning Microscopy (TSM). In addition to these, I will also discuss relevant related techniques and equipment used to measure tooth wear and DH.

1.15.1 Clinical indices to measure tooth wear

Tooth wear may be directly observed at the chair side (El Aidi *et al.*, 2008) or recorded using study casts (Bartlett, 2003) and progression may then be measured (Bartlett *et al.*, 2005), although this process is highly subjective with great inter- and intra- operator

variability. It is also possible to use sequential study models or silicone putty matrices that give an indication of tooth wear progression over time and in more detail in the laboratory using metrology systems, which measure surface topography (Rodriguez *et al.*, 2012; Suga, 2007). Metrology is discussed in more detail in section 1.21 on profilometry. However, profilometry requires considerable time to measure each tooth (>1 hour) and is unsuitable to measure wear and its progression in large populations (Bartlett *et al.*, 2011a). Furthermore, tools used to measure DH clinically require in conjunction an index for tooth wear, which is easily measured and allows clinical comparison and at the same appointment.

For the purpose of measuring the prevalence of tooth wear in the community, tooth wear indices have been developed. They have also been used to diagnose, grade and monitor tooth wear caused by attrition, abrasion and or erosion (Bardsley, 2008). Some indices record lesions on an aetiological basis (e.g. erosion indices), whereas others record lesions irrespective of aetiology (tooth wear indices). A number of indices have been proposed and are summarised in Table 5. However, none have universal acceptance, perhaps due to a lack of standardisation in their terminology and/or vague definitions of their criteria used to grade tooth wear, which mean that interpretation of severity scores is not clear-cut (Bardsley, 2008).

Table 5 Tooth wear and erosion indices adapted from (Bardsley, 2008)

Tooth wear index	**Author**	**Purpose**
Anthropological studies of dental systems	(Broca, 1879)	Grade horizontal or oblique patterns of occlusal wear irrespective of cause.
Six point grading	(Restarski *et al.*, 1945)	Evaluation of the severity of erosion

Tooth wear index	Author	Purpose
system		on the lingual surfaces of rat and puppy molars.
Eccles tooth wear index	(Eccles, 1979)	Grading the severity and site of erosion from non industrial erosion.
Dental erosion index	(Xhonga and Valdmanis, 1983)	Grading the severity of erosive lesions using a periodontal probe and differentiation of the type of erosion by morphology.
Tooth wear index (TWI)	(Smith and Knight, 1984b)	Graded tooth wear irrespective of cause.
Qualitative Index	(Linkosalo and Markkanen, 1985)	Erosive lesions diagnosed and severity graded leading to involvement of dentine.
Tooth wear index	(Oilo *et al.*, 1987)	Used to evaluate tooth wear and the need for treatment.
Clinical erosion index	(Larsen *et al.*, 2000)	Record and evaluate erosive wear clinically and on study casts.
Simplified TWI	(Bardsley *et al.*, 2004)	Grading tooth wear in epidemiological studies.
Basic Erosive Wear Index	(Bartlett *et al.*, 2008)	Screen erosive wear in dental practice and epidemiological studies.
Exact tooth wear index (ETI)	(Fares *et al.*, 2009)	Grading severity of tooth wear in enamel and dentine.
Visual Erosion Dental	(Mulic *et al.*, 2010)	Diagnose early stages of erosive

Tooth wear index	Author	Purpose
Examination		wear and to record progression on an individual basis.

An early index for erosive wear (Restarski *et al.*, 1945) averaged the tooth wear score from all teeth, but its criteria definitions were vague. Also, the score was calculated by summation of the mean molar quadrant scores. This could underestimate the level of tooth wear for example in the case of a localised advanced wear. The Eccles index (Eccles, 1978) was then designed to measure wear caused by erosion and is considered one of the cardinal indices from which others have evolved (Bardsley, 2008). Eccles first classified erosive wear as early, small and advanced with no strict criteria (Eccles, 1978), but to help with interpretation it was later described in more detail, grading both the severity and site of erosion. Erosion is based on three classes denoting the type of lesion, assigned to four tooth surfaces (Eccles, 1979). It recorded erosion on the buccal/facial, occlusal and palatal tooth surfaces in a referred population using the terms Class I, II, IIIa, IIIb, IIIc and IIId. The Eccles Index was very complicated in particular for the measurement of advanced wear. Perhaps in an attempt to describe the severity of erosive wear more accurately, a periodontal probe was also used in a later study to measure the size of the lesion and divide erosive lesions into four levels, but inter- and intra-examiner variability was not investigated (Xhonga and Valdmanis, 1983).

An early index to measure tooth wear for use in general dental practice was first described by Smith and Knight and developed from the Eccles index (Smith and Knight, 1984b). The Smith and Knight Tooth Wear Index (TWI) classifies tooth wear on a five point scale based upon observation of severity of tooth wear with a threshold set

for dentine exposure. It is taken at four sites per tooth (cervical, buccal, occlusal/incisal and palatal/lingual). Using this scale, wear in dentine has three grades, whereas wear in enamel has one grade. This is because it aimed to assess the need for operative intervention, with focus on severe dentine involvement, and less focus on changes at the enamel level. A score of zero indicates no wear and a score of four indicates wear at or near to pulp exposure. The TWI standardised all forms of tooth wear clearly and for training purposes. By comparing with threshold values for the age group studied, it was also able to distinguish pathological and physiological tooth wear (a description of pathological and physiological tooth wear is included in section 1.5). Like the Eccles index, the TWI similarly records wear on the buccal/facial, occlusal/incisal and palatal/lingual tooth surfaces, but without the class divisions of the Eccles index. However, the TWI is descriptive and not based on a diagnosis of aetiology. TWI thus avoided problems caused by differences in opinions with regards to the aetiology, as it did not code based on cause/s or treatment need. Interestingly, this feature of the TWI is similar to one of the earliest reported tooth wear indices (Broca, 1879).

Smith and Knight used their index in a series of investigations, which reported the prevalence of tooth wear in a population referred to a dental school and also in a study conducted in general dental practice (Smith and Knight, 1984a; b; Smith and Robb, 1996). Inter- and intra-examiner reproducibility using the TWI was acceptable. The confidence of the Smith and Knight within the dental profession is reflected by its use in a number of studies investigating the prevalence and severity of tooth wear (Bartlett *et al.*, 1998; Lussi *et al.*, 1991; Milosevic *et al.*, 1994; Poynter and Wright, 1990) aetiology and risk (Asher and Read, 1987; Milosevic *et al.*, 1997) or using modifications to study the primary and secondary dentition (Millward *et al.*, 1994) or elderly populations (Steele *et al.*, 1996). In the latter study, it was modified by combining lower tooth wear scores and by recording the worst surface score per tooth as an overall tooth wear

score (Steele *et al.*, 1996). It was also suggested in another study that TWI be expanded to reflect an aging population and a more severe wear (such as pulpal exposure), which may then occur (Donachie and Walls, 1995; 1996). TWI was also used as a basis for the 1993 children's dental health survey (O'Brien, 1993) and for subsequent studies of children (Dugmore and Rock, 2004; Harding *et al.*, 2003; Harding *et al.*, 2010; Whelton *et al.*, 2008).

One problem with the Smith and Knight TWI is that it only has one score for enamel and it therefore under-represents changes to the enamel and restricts use of these indices for interventional studies on prevention as most changes in tooth wear begin with enamel (Fares *et al.*, 2009). Despite the aim of the TWI to record the restorative needs of a population, it was biased towards severe forms of dentine exposure and of little use for early wear in enamel or to investigate the effect of preventive treatments (Bartlett *et al.*, 2011a). Complete enamel loss may not occur because, as is often the case in erosive lesions, a rim of enamel at the worn surface often remains known as the 'enamel halo'. Another problem with Smith and Knight is that it is a complicated index, takes considerable time to apply to the entire dentition and generates a lot of data. It was likely to over-estimate the amount of tooth wear by asking the examiner to estimate the proportion of teeth affected by tooth wear (Ganss *et al.*, 2006). Another problem was that the threshold levels at each age group for which tooth wear was compared to were high, hence pathological tooth wear could often be underestimated (Bardsley, 2008).

Further indices were then developed to qualify tooth wear as erosive using diagnostic criteria and relating to the involvement of dentine (Linkosalo and Markkanen, 1985). These were modified to create the erosion index according to Lussi (Lussi *et al.*, 1991). The index graded tooth wear lesions based on the surface (facial or occlusal/lingual)

and using a scoring system describing the morphological characteristics of the lesion and the presence of dentine. Using the Smith and Knight, further indices were also developed to measure dental erosion clinically and on study casts, which were kept for the purposes of monitoring tooth wear over time (Larsen *et al.*, 2000). However these were complicated and difficult to use. All the indices so far include a number of grades to measure tooth wear. However, for the purposes of epidemiological studies on large numbers of subjects, a simpler approach was proposed by Bardsley based on the Smith and Knight TWI and used to measure wear on 40 tooth surfaces per subject (Bardsley *et al.*, 2004). Known as the simplified TWI, it is based predominately on the degree of tooth wear in dentine and is shown in Table 6.

Table 6 Simplified TWI

Score	**Description**
0	No wear into dentine
1	Dentine just visible (including cupping) or dentine exposed for less than 1/3 of surface
2	Dentine exposure greater than 1/3 of surface
3	Exposure of pulp or secondary dentine

Nonetheless, another study discussed the difficulties in diagnosing exposed dentine despite calibration and training and one study showed that correlation between two examiners using visual diagnosis of exposed dentine is poor (Ganss *et al.*, 2006). It had been recognised that studies should include identification of tooth wear at an early stage (Dugmore and Rock, 2003). In order to reduce the under-representation of the enamel as could often occur in the earlier tooth wear indices such as Smith and Knight, the Exact Tooth Wear Index (ETI) was developed in part from the Eccles index (Eccles, 1979). It recorded tooth wear in enamel and dentine at a more detailed level than earlier indices and included a four-point scale for enamel wear and a five point scale for dentine wear. It was used in a study on 1, 010 University college students aged 18-30 years old in London, UK (Fares *et al.*, 2009) and another study on 123 children aged

12 (±0.32) years in Cork, Ireland. The ETI index aimed to create easily understood clear scoring criteria, be reproducible and inform research on prevention and monitoring of tooth wear for research purposes. Inter-examiner agreement was also evaluated and shown to be greater than 0.8 (Fares *et al.*, 2009). However, due to a number of grades for both enamel and dentine wear, this index may not be convenient to measure tooth wear routinely in general dental practice on a larger number of patients in epidemiological studies. In addition, these indices also relied on differentiation of lesions localised to enamel or dentine especially in the cervical area of teeth and the difficulty in doing this has been discussed (Ganss *et al.*, 2006; Holbrook and Ganss, 2008).

The Basic Erosive Wear Index (BEWE), first described by Bartlett *et al.* (Bartlett *et al.*, 2008) was developed as a practical screen for scoring erosive wear in general dental practice using a sextant cumulative score. It is a useful and quick tool to periodically screen tooth wear and can be made more reproducible following calibration training. The index was based on the Basic Periodontal Examination (BPE), which has been widely used in general dental practice and is also sextant based (Smales *et al.*, 1987). The BEWE sextant score provides a guide to risk and aims to increase awareness amongst clinicians of a patient's level of tooth wear and may also help to guide clinical management. It involves a visual examination of all the teeth (excluding third molars) and records the damage done to the teeth using a four point ordinal scale. The scale ranges from 0 to 3 (0=no erosive wear, 1=initial loss of surface texture, 2=hard tissue loss, less than 50% of surface area (clinical crown), 3=hard tissue loss more or equal to 50% of the surface area (clinical crown)). The highest score is then recorded in each sextant intra-orally and the total score provides an indication for management (Bartlett *et al.*, 2008). The BEWE removes the clear distinction between enamel loss and dentine exposed and the difficulties involved in differentiating the two as discussed

previously (Bartlett *et al.*, 2008; Ganss *et al.*, 2006). The risk score includes a focus on prevention in management strategies and also relates to previous scoring systems for the purposes of specific research purposes. However, due to its sextant design, it requires training, calibration and validation to ensure it is providing a fair representation of tooth wear on all teeth.

To date, only one study has looked at the reliability of the BEWE scoring system (Mulic *et al.*, 2010). In this study, the BEWE and the Visual Erosion Dental Examination (VEDE) were investigated. Inter- and intra-examiner agreement of the BEWE and VEDE indices were obtained and found to be similar. The VEDE has two scores for enamel loss and three scores for dentine loss and despite diagnostic uncertainties regarding dentine diagnosis, differentiation between enamel and dentine may be an important factor for recording the progression of tooth wear and dental erosion (Fares *et al.*, 2009; Ganss *et al.*, 2006). However, this study only reported erosive wear and the VEDE measured erosive wear at the tooth surface level, not as a cumulative score per patient. Moreover, no direct statistical comparison was made between both systems. The BEWE although examining all teeth could be considered a partial, as opposed to full mouth scoring instrument because the multiplicity of sites is not considered when a single score is applied per sextant. No attempt has been made to validate the BEWE sextant cumulative score (giving the overall risk) with a BEWE score taken from all tooth surfaces. This is necessary for diagnostic, management and research purposes.

For completion, it should also be noted that other indices have been described based on treatment need. These have used a system based on dentine exposure and clinical findings such as pain, sensitivity and fracture of restorations (Oilo *et al.*, 1987). They have been further developed with more categories but are complex and poorly

investigated. It is important for studies to be consistent in reporting tooth wear data, for example either as the proportion of surfaces affected by wear (Dugmore and Rock, 2004) or as the proportion of subjects with wear (Bardsley *et al.*, 2004). It was recommended in review that both surface and subject measures be used in order to ensure studies can be compared (Bartlett *et al.*, 2011a).

1.15.2 Clinical studies on DH

Clinical studies may be conducted to measure qualitatively the presence/absence of DH symptoms reported by subjects and therefore can also be used to investigate the effects of desensitising dentifrices that use nerve depolarisation and/or dentine tubule occlusion. Guidelines were published in 1997 for the purpose of obtaining consensus for the design and conduct of clinical trials on DH in response to abundant previously published research papers, which had used highly variable protocols (Holland *et al.*, 1997). The guidelines were written by a committee; comprising academics, clinical specialists and industrial representatives, who all had an interest and expertise in DH and clinical trials. It discussed solutions for key questions on sensitivity testing, which had been raised in a previous paper (Orchardson *et al.*, 1994) and included experimental design, sample size, subject and tooth selection, controls, duration, assessment and follow up. Typical subject exclusion criteria adopted in clinical studies investigating desensitising dentifrices include;

1. Current desensitizing therapy,
2. Medical (including psychiatric) and pharmaco-therapeutic histories that may compromise the protocol – including the chronic use of anti-inflammatory, analgesic and mind-altering drugs,
3. Pregnancy or breast feeding,
4. Allergies and idiosyncratic responses to product ingredients,

5. Eating disorders,

6. Systemic conditions that are etiologic or predisposing to dentine hypersensitivity (e.g., chronic acid regurgitation),

7. Excessive dietary or environmental exposure to acids,

8. Periodontal surgery in the preceding 3 months (unless it is the effect of the agent on post-surgical sensitivity that is under study),

9. Orthodontic appliance treatment within previous 3 months,

10. Teeth or supporting structures with any other painful pathology or defects,

11. Teeth restored in the preceding three months,

12. Abutment teeth for fixed or removable prostheses,

13. Crowned teeth,

14. Extensively restored teeth and those with restorations extending into the test area.

It was also recommended by Holland *et al.* that there be a run-in/wash-out period during which the study population are prohibited from using the de-sensitising products and their home care regime is standardised, for example by providing all participants with a standard toothpaste and brush.

For the purposes of measuring DH *in vivo* from patient based responses, it was recommended that tactile, cold or evaporative air stimuli may be applied to the teeth as both are physiological and controllable (Holland *et al.*, 1997). It was suggested that at least two stimuli be used per subject because subject reported-pain-thresholds vary between stimuli. Also, it was proposed that an interval was included between stimulus applications and of sufficient time to minimise potential interactions between stimuli. This paper also recommended that ideally the same investigator should evaluate each subject in a trial and most importantly using the same stimulus. If multiple investigators

are used, they should be uniformly calibrated. The number of stimuli, which have been used previously in eliciting DH, is vast. Table 7 lists several stimuli used, adapted from (Gillam *et al.*, 2000).

Table 7 Stimuli used to assess DH clinically

Routinely used	Less commonly used	Not recommended
Constant pressure probe (Yeaple), Evaporative stimuli or cold air stimuli from a dental air syringe.	Explorer probe, mechanical pressure stimulators and tactile stimuli Scaling procedures, Single-tufted brush, Chemical (osmotic) stimuli, Hypertonic solutions such as sodium chloride, glucose, sucrose, or calcium chloride, Cold water testing, Thermal stimuli Thermo-electric devices (e.g. Biomat Thermal Probe, London, UK), Ethyl chloride Ice-stick, Temptronic device (microprocessor temperature-controlled air delivery system), Dental pulp stethoscope, Air jet stimulator, Yeh air thermal system.	Electronic threshold measurement device, Electrical Stimulation, Electrical pulp testers.

For tactile stimuli, the Yeaple probe is often used. This is an electronic force-sensitive probe, originally used to measure the periodontal pocket depth. It has also been used to manually apply a predetermined probing force to exposed dentine by running the tip of the probe across the tooth surface. The force setting can be set at 10 g and increased to 50 g until the subject reports a feeling of pain. The force assessment can then be repeated a second time to confirm the assessment (Curro *et al.*, 2000). The tooth surface generally scores as being sensitive, if the subject reports pain on both consecutive occasions. Higher levels of sensitivity correspond with lower force settings. This technique has been used in clinical trials comparing products to manage DH (Mason *et al.*, 2010). Dental explorers, force probes or 'scratchometers' may also be used as tactile stimuli to ascertain DH. However, these have been contraindicated for the purposes of evaluating adhesive restorations or other barrier methods (Holland *et al.*, 1997) perhaps as the stimulus could disturb the protective surface covering.

Evaporative air sensitivity assessments are most commonly conducted using air directed from a triple air dental syringe. One clinical trial (Mason *et al.*, 2010) used air at 60 psi (±5 psi) and 19°C (±5 °C) directed at the exposed dentine surface from a distance of approximately one cm for one second. The presence of DH may then be assessed using an examiner-based assessment known as the Schiff cold air sensitivity scale (Schiff *et al.*, 1994) or using a subject based assessment such as the Visual Analogue Scale (VAS).

The Schiff index records DH on an ordinal scale as;

0= Subject does not respond to air stimulus,

1= Subject responds to air stimulus but does not request discontinuation of stimulus,

2= Subject responds to air stimulus and requests discontinuation or moves from stimulus,

3= Subject responds to the air stimulus, considers stimulus to be painful and requests discontinuation of the stimulus.

The VAS involves rating the intensity of the pain response on a 100mm line, ranging from ‘no pain’ (on the left hand side) to ‘very severe pain’ (on the right) as shown in Figure 2.

Please place a vertical mark on the line below to indicate how bad you feel your dental sensitivity is today:

No pain _____ Very severe pain

Figure 2 Visual analogue scale

For the purposes of eliciting DH, incisors and premolars have been proposed for use, with molars less so (Holland *et al.*, 1997). Since (Holland *et al.*, 1997), a number of clinical trials have been used to investigate the efficacy of products, including desensitising dentifrices, which are used in the management of DH. However, disadvantages have been realised. First, it was recommended by Holland that subjects should have sensitive teeth and satisfy the definition of DH. Clinical trials therefore rely upon an often very large number of subjects all presenting with DH at the start of the trial. However, an innate problem is that subjects do not always present with DH and there are increasing, albeit often anecdotal, suggestions from the literature that the nature of DH is episodic (West, 2006). For the purpose of conducting clinical trials on DH requires strict standardisation of methodologies, exclusion and inclusion criteria in addition to the control of lifestyle, diet and other factors, which might be associated with DH. Removing all these sources of alone error could be impossible. To date, no large clinical studies have investigated the presence or otherwise absence of DH in association with its various aetiologies, to validate the episodic nature of DH clinically.

Other disadvantages in clinical trials are that there can be difficulty in the actual measurement of sensitivity clinically. This includes large variability in the subject-based reproducibility of DH and pain tolerance despite standardisation of stimuli used to elicit DH (Ide *et al.*, 2001) and the Hawthorne effect in controlled clinical studies (West *et al.*, 1997). Furthermore, clinical trials do not provide information on the mechanism of action of DH.

To remove subjectivity and sources of error requires a method of measurement and standardisation beyond those used in clinical trials alone. For the investigation of desensitising dentifrices, this may involve visualisation of the surface characteristics of dentine. Ideally, this would take place *in vivo*, however to date most studies have been conducted either *in vitr*o or *in situ*.

1.16 Laboratory studies on tooth wear and DH

Laboratory investigations are commonly undertaken in addition to clinical investigations of therapeutic agents used to treat or prevent DH and tooth wear. Most studies on tooth wear and DH have been conducted *in vitro using* dentine discs, derived from teeth sectioned near the cement-enamel junction (CEJ) then ground and polished (Absi *et al.*, 1995). They can then be subjected to various wear regimes and tooth wear may be measured using surface metrological measurements including profilometry, electron microscopy, micro-hardness or confocal microscopy. In the laboratory, DH is measured indirectly for example from the presence or absence of un-occluded dentine tubules microscopically or using hydraulic conductance to measure permeability. Microscopic techniques include a range of electron and confocal microscopy techniques and energy dispersive x-ray microscopy to measure the chemical nature of materials used to

occlude the dentine tubules. Some techniques would preclude imaging on patients directly due to their damaging effect on live tissue. As discussed in section 1.1.1, the most important anatomical factor in determining fluid flow within dentine tubules and hence the hydrodynamic theory is the diameter of the dentine tubule raised to the fourth power (Pashley, 1990c). With this in mind, it has been demonstrated that microscopic techniques can be very useful to measure the amount of patent or un-occluded dentine tubules (Absi *et al.*, 1987). However, the results of *in vitro* studies should be interpreted with caution, as they may not always be replicated clinically, due to the effect of the oral environment *in vivo* (Ling *et al.*, 1997).

1.17 Electron microscopy

Electron microscopy works by bombarding the surface of a sample with an electron beam. The electrons are either displaced from the surface of the sample as secondary electrons for Scanning Electron Microscopy (SEM) or transmission of the electrons through the specimen for Transmission Electron Microscopy (TEM).

1.17.1 Scanning electron microscopy (SEM)

SEM is able to image the surface of a sample using a large depth of field and a high resolution (of up to 1 nm) using a field emission system and in-lens detector. The resolution will depend upon the dimension of the probe beam, diffraction at the final aperture, chromatic aberration and the size of the electron source. The micro-structural appearance of material and hard tissues may be appraised quickly and extensively. An SEM can obtain images of up to x400, 000 or hundreds of nanometres, but this is determined by the excitation of the scan coils, which could be affected by residual

magnetic or stray fields. Magnification is also affected by working distance between the lens and sample (Egerton, 2008; Goodhew *et al.*, 2000).

Biological samples must be processed in order to obtain a vacuum in the sputter coater and microscope chamber. This involves various post-fixation drying techniques on all samples, which may affect the surface characteristics of the specimen. Studies have been conducted to study the effects of various sample-processing techniques. In one study, four post-fixation drying techniques on etched dentine were compared. These included; critical point drying, hexamethyldisilazane drying, Peldri II drying or air-drying. Each processing technique showed considerable differences in the appearance of dentine examined using SEM (Perdigao *et al.*, 1995). Another study investigated the effects of drying dentine samples in preparation for SEM on the diameter of the dentine tubules. Dentine tubule diameters were quantified using light microscopy and SEM at various points post sample demineralization and air-drying. They showed that the diameter of the tubules decreased significantly following dehydration (Arends *et al.*, 1995). Following dehydration, the samples are then placed in a vacuum in a sputter coater. Here, each sample is coated with a thin (15-20 nm) metallic (gold, gold-palladium or platinum) or carbon layer to prevent and dissipate the build-up of electric charge on the sample during the electron bombardment. Shrinkage of the dentine samples occurs following dehydration, vacuum and coating because dentine contains 10% by weight (and 20% by volume) water.

In order to overcome the problems associated with dehydration shrinkage, freeze-drying techniques have been used prior to coating, although contraction stress is still likely. A second approach is to use replication impression techniques, in which a silicone impression is used to obtain a negative replica of the sample or an epoxy resin positive replica is then cast from the impression to help in interpretation. It is then

sputter coated and viewed using the SEM (Barnes, 1978). This also has application for *in vivo* studies (Weber, 1983). Similar replica impression techniques have since been developed to investigate dentine tubule occlusion *in vivo* or the effects of desensitising dentifrices on dentine tubule occlusion *in situ* (Absi *et al.*, 1989; Claydon *et al.*, 2009). Another study found that the replica impression technique has use for recording the fluid outflow from dentine tubules (Kerdvongbundit *et al.*, 2004). However, replica impression techniques do not provide a direct image of the dentine surface. Secondly, each sample relies on two impressions in order to create a positive replica of the dentine surface. This introduces errors due to the impression technique, the operator and shrinkage of the impression material. Kerdvongbundit *et al.* 2004 mentioned that the impression might also be affected by drying of the dentine surface. Lastly, sputter coating of the resin replicas could cause the replicas to melt if the sputter coater were to overheat.

Despite the dehydration and processing stages involved in SEM, an advantage of this compared to other experimental techniques (such as those described in sections 1.19 and 1.20 on 'Confocal microscopy' and 'Hydraulic conductance') is that samples can be re-examined by any investigator and may be stored. This provides a database of samples that can be accessed by future researchers for reference, calibration and training (Ahmed *et al.*, 2005). It is also a standard technique for investigating dentine tubule occlusion and has been widely used to visualise dentine tubules and the surface of dentine in order to investigate the effects of factors such as erosion, abrasion and attrition and desensitising dentifrices used to treat DH (Ahmed *et al.*, 2005). It therefore serves as a useful baseline to which other techniques can be compared. It is also important that the imaging and processing technique is standardised to reduce variability.

Charging is due to the buildup of excess electrons on the surface of the sample and is the greatest impediment to the quality of the SEM image (Rice, 2012). Of the electrons which hit the sample, some of these are secondary or back scattered and generate useful electrons for imaging. The remainder leaves the electrical ground of the SEM. The buildup of electrons or charge creates an electric field which deflects the electron beam in an undesirable way. An example of such an image is shown in Figure 3. This shows some of the un-occluded dentine tubules are surrounded by a bright halo.

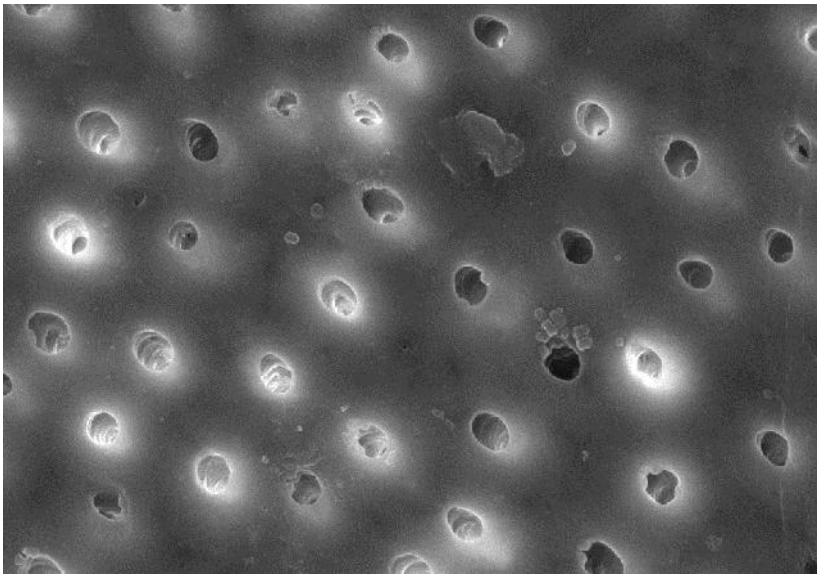


Figure 3 Charging effects on SEM images

In order to overcome charging of SEM images, a number of options have been described (Rice, 2012). These included;

- Coating the sample with a conductive layer (i.e. gold coating),
- Reducing the voltage to reduce the sample of electrons,
- Reducing the spot size or beam current to reduce the number of electrons hitting the sample,
- Reducing the vacuum in the SEM chamber so that gas in the chamber absorbs some of the electrons.

Following imaging, various methods have then been used to measure dentine tubule occlusion. These have included a visual count of all dentine tubules on each image, or using a grid based system to count dentine tubules on certain areas of the sample, or using a semi-quantitative visual scale (Hooper *et al.*, 2005; West *et al.*, 1997; West *et al.*, 2002). All require training of examiners. These various systems will be discussed in more detail in section 1.23.

Environmental SEM (ESEM) allows dental hard tissues to be visualised under a variety of atmospheric conditions, thus avoiding the dehydration and sputter processes used during conventional SEM techniques. Therefore hydrated samples may be studied because the microscope contains a pump, which ensures a vacuum whilst maintaining control of the water vapour pressure. It has been used *in vitro* to compare the effects of various treatments on dentine samples and the effects of further post treatments (such as acid erosion) on the same dentine samples periodically (Wang *et al.*, 2010). It is likely to have further potential for *in situ* studies, to allow the non-destructive re-observation of dentine samples. However, ESEM does not allow samples to be imaged in completely wet conditions (Kubinek *et al.*, 2007) and to date no randomised control studies have been conducted *in situ* to measure dentine tubule occlusion using ESEM.

1.17.2 Transmission Electron Microscopy (TEM)

TEM involves transmission of electrons within a specimen and requires thin, fixed and embedded specimens to withstand overheating or destruction in the vacuum and electron bombardment. It has a resolution to tens of nanometres and has been used to study the crystallite size and mineral content within dentine (Porter *et al.*, 2005). Clearly, it images sub-surface and therefore its use for visualising surface particulate deposits, which may occlude dentine tubules, is limited.

1.17.3 Focused ion beam scanning electron microscopy (FIB-SEM)

FIB can be used to mill away volumes of material, in user defined areas, to produce cross-sectioned specimens, which can be used in subsequent SEM and TEM and for chemical analysis (Earl *et al.*, 2010). FIB might reduce the generation of sample preparation artefacts that are easily created for example if using a microtome to cross section a sample (Earl *et al.*, 2010a). The FIB-SEM equipment consists of a focused beam of charged particles and a secondary beam containing electrons. Both the ion and electron beam may be used for the purposes of imaging, but due to the damaging effect of the ion beam, the electron beam is generally used. The technique has been used to section dentine tubules in order to investigate sub surface the presence of dentine occlusion within dentine tubules (Earl *et al.*, 2010b). However, it should be noted that the sample requires a number of processing stages, the ion beam might damage the dentine surface (Langford, 2006) and a gold or carbon coating may be required to limit the damaging effect of the beam (Bender, 1999).

1.18 Energy Dispersive X-ray spectroscopy (EDX)

Energy Dispersive X-ray (EDX) spectroscopy is an analytical technique for the elemental analysis or chemical characterisation of a sample, by analysing x-rays emitted by the sample in response to being hit by charged particles (often an electron beam from an SEM). The energy dispersive spectrometer then measures the number and energy of x-rays emitted from a sample, which are characteristic of an elements atomic structure and a spectrum is created.

Accuracy can be affected by over voltage, and difficulty identifying elements with overlapping peaks on the same spectrum. In addition, for rough or inhomogeneous samples (such as dentine treated with dentifrice), x-rays may be less likely to escape the sample and be detected. Nonetheless, EDX has been successfully used previously to identify the nature of occluding deposits in dentine tubules and in combination with FIB (Earl *et al.*, 2010b; Suge *et al.*, 2005).

1.19 Confocal microscopy

High-resolution reflected light optical microscopy or confocal optical light microscopy allows the study of dental hard tissue, soft tissue and materials (Watson and Boyde, 1991; Watson, 1997). An advantage is that it avoids sample processing in preparation for imaging unlike other forms of microscopy, such as conventional light, electron and x-ray micro-radiology and the sample can be studied in as close to its natural state as possible.

Confocal means “having the same focus”. The confocal microscope has an aperture in the conjugate focal plane of an objective lens in both the illumination and imaging pathway. Objects in the focal plane of the objective are illuminated by a point source and the light reflected by the specimen is seen by a point detector. The two apertures are aligned such that light from the source aperture passes through the viewing aperture and so that the reflected light originates from the same place of focus within the specimen. Unlike a conventional light microscope, light from above and below the confocal plane is not allowed to pass through the detecting aperture. This results in an image of high resolution and contrast. The resolution is in-between that of a conventional light microscope and SEM or TEM (Watson and Boyde, 1991).

Confocal imaging allows high-resolution real time investigations of a surface. In a process termed optical sectioning, the focal plane may also be moved in the y-axis to allow sub-surface imaging of thin optical sections in a translucent sample. This occurs in real time using specially set up optical systems. Using this technique, it is possible to image thin slices greater than 35μm, which are up to 200μm sub surface. For enamel and dentine, it is probable that slices greater than 1μm to a maximum depth of 100μm into the sample can be imaged (Watson, 1997). These images are then capable of 3D reconstruction of the sample (Watson, 1991). For example, summation of images taken up to 100μm within enamel or dentine using a special immersion media allow peak to trough (z height) measurements to be taken in order to provide information such as average roughness (ra) of the surface of a sample (Watson, 1997). However, Watson explains that interpretation of these reconstructions should be undertaken cautiously because resolution in the optical axis of the microscope is less. It is helpful to have a flat tooth surface as a reference point for sub-surface imaging. However, some immersion media such as oil are likely to render samples unusable for subsequent experimental investigation.

Immunohistochemical labelling may also be used to provide fluorescent imaging and help enhance images obtained using confocal microscopy (Watson, 1997). Fluorophores or fluorescent dyes are added to samples to make them fluoresce. In similarity to subsurface imaging, the disadvantage of this technique is that it requires some sample preparation. In addition, many of the dyes used for florescent imaging are acidic, because they consist of proteins containing aromatic amino acid residues that might contribute to their intrinsic fluorescence.

There are two types of confocal microscope; Laser Scanning Confocal Microscopes (LSM) and Tandem Scanning Confocal Microscopes (TSM). The LSM are best suited

to fluorescent imaging. The TSM are best suited to high-speed surface imaging of dentine samples *in vitro* and have also been used for *in vivo* imaging of dental structures at high resolution (up to x240). However, difficulties may arise, *in vivo* due the sample stabilisation required at high resolution (Watson *et al.*, 1992).

1.19.1 Tandem Scanning Microscopy

Tandem means “working in conjunction”. Scanning for the purposes of TSM means to move a finely focused beam of light over a sample in a systematic pattern. An integral component of the TSM is the scanning disc, which is contained within the confocal system. The disc is perforated with thousands of tiny apertures (<60μm in diameter) and arranged in a pattern, which is symmetrical about any diameter. It is two sided and is rotated at 100-150 revolutions per minute. This illuminates apertures (>1000) on one side of the disc, which act as the light source. The conjugate apertures on the other side of the disc are the point detectors; through which reflected light from the sample passes. The disc rotates in the intermediate image plane of the objective and this scans the field of illumination across the sample surface (less than $1/20^{th}$ of a second). This creates a real time image of the surface of the sample, with only light reflected from the sample surface being used to create a true colour image. The optical paths of the illuminating and reflected light are separated such that the brighter illuminating light does not obscure the low light levels of the light reflected from the sample (Pawley, 1995). The frame rate can also be adjusted and in addition various filters can be used to adjust the image contrast.

A ‘one sided’ or unilateral TSM was developed in 1987 in which light is transmitted through the same pinhole in the scanning disc. This reduces problems in ensuring the pinholes are always aligned. However, stray reflected light must be removed using

other components and this could potentially affect the contrast and resolution of the image. Unilateral TSM and bilateral TSM can be used in both the confocal or standard microscope modes, but for bilateral TSM requires removal of the entire disc. Both can also be used for image video recording in real time using a silicone intensified target camera (SIT) or a charge-coupled device camera (CCD); a cooled CCD is more effective for TSM because there is less quantity of light reflected back through the pinholes in the disc. However, fluorescent imaging is easier using bilateral TSM (Boyde *et al.*, 1990). The direct real time imaging requires an increased disc rotation speed of >30 frames/second.

The confocal microscope used in this study is a double-sided scanning disc confocal microscope, the TSM (Tracor Northern) confocal microscope, with a mercury vapour illuminating source. This light source contains the full spectrum of wavelengths required for real-colour imaging, unlike in confocal laser microscopy. However as eluded, the total light efficiency of the TSM is small (only 1-2%) and maximising this is therefore important. Increasing the pinhole size will increase the illumination, but this will also affect the resolution. This can be improved in fluorescent imaging by using stronger ‘fluorophores’. Mechanisms of altering image brightness are shown in Figure 4.

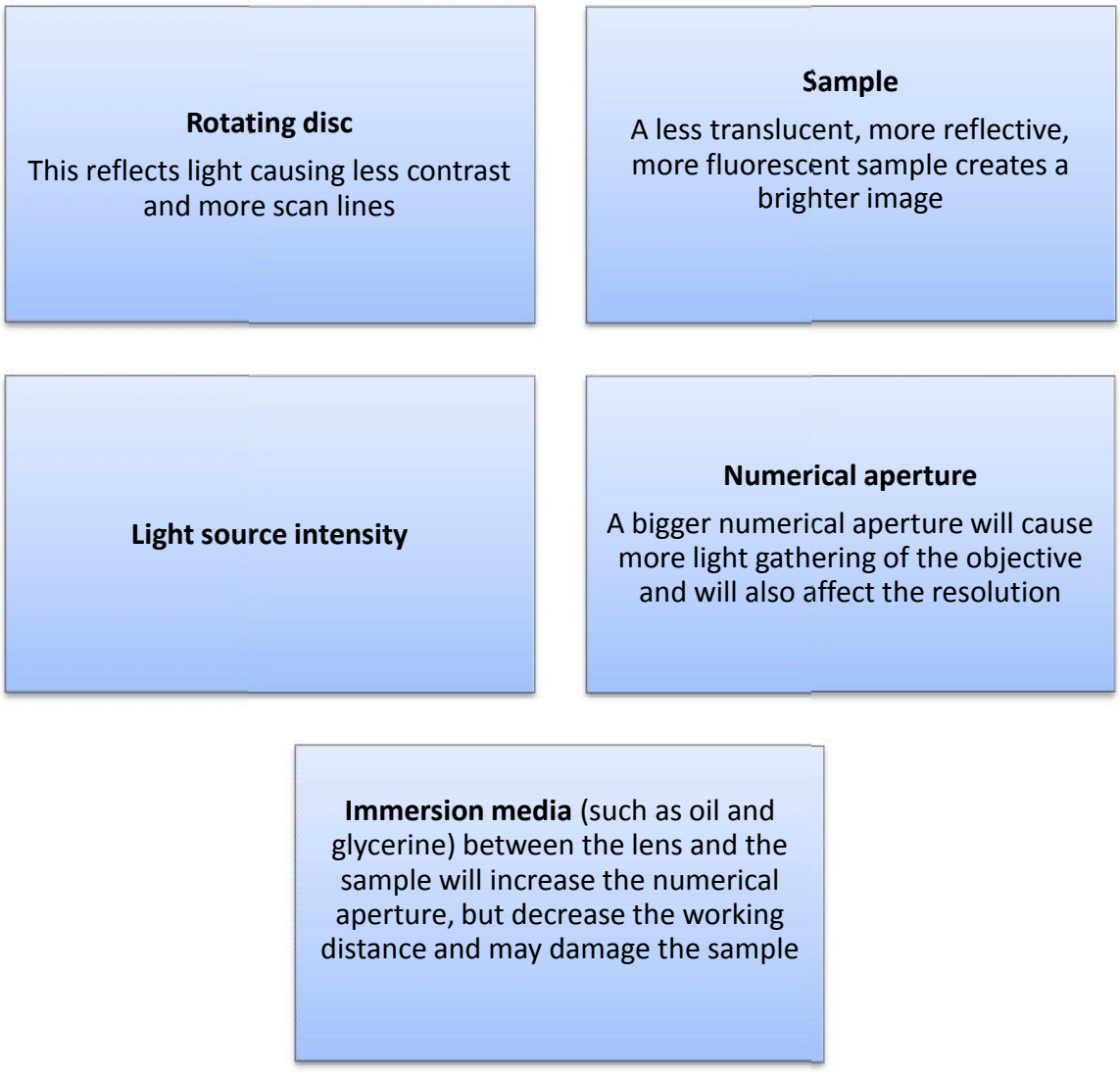


Figure 4 Factors affecting image brightness in confocal microscopy

1.19.2 Confocal Scanning Laser Microscopes (CSLM)

Rather than white light, these microscopes use a laser beam to scan the sample. This beam originates from a black box within the head of a conventional light microscope. The size of the point detector can be adjusted in order to adjust the confocal imaging. In addition, they are often used for immunofluorescence imaging using fluorescent dyes. When these dyes are irradiated, part of the energy of the incident radiation is

then emitted as light normally of a longer wavelength than the source. The advantage of the CSLM over the TSM is that a small volume of fluorophore is required because the incident radiation is high energy.

The first designs of CSLM moved the specimen under a stationary beam and were limited by the rate of movement of the specimen under the beam (with a frame rate of minute's duration). This was overcome using galvanometer mirrors to move the beam across the specimen. Furthermore, acoustic-optical deflection devises may be used to move the scanning beam and produce images at TV frame speeds (Draaijer and Houpt, 1988). CSLM has been used to compare products used to occlude the dentine tubules using an oil immersion and sub-surface imaging to create z-stack projections *in vitro* (Sauro *et al.*, 2010). Not all CLSM use immersion however it should be noted that the oil based immersion medium would destroy samples and preclude further use. The LEXTOLS4000 3D laser-measuring microscope is a typical example, which requires no sample preparation. Nonetheless, sub surface images alone may not reflect surface particulate deposits on the samples that may create an occlusion.

1.20 Hydraulic conductance

The hydrodynamic theory, described in section 1.1.1, is based upon the premise that sensitive dentine is permeable and that dental nerves are functioning properly. Increases in the rate of fluid flow or in the nerve excitability could cause an increase in DH. The hydraulic conductance (Lp) of a tissue, such as dentine, expresses the ease to which fluid can move across a unit surface area under a unit pressure per unit of time (Pashley, 1990b). Hydraulic conductance depends upon the pressure of fluid movement across dentine, length of the dentine tubules and viscosity of the fluid and

radius of the dentine tubule (Pashley, 1990b), with radius raised to the fourth power being the most important factor.

The apparatus for measuring dentine permeability using hydraulic conductance was first described for the purposes of investigating the effect of adhesives on the permeability of dentine (Pashley *et al.*, 1988; Prati *et al.*, 1991). The typical apparatus is shown in Figure 5. It has since been used in laboratory studies to investigate the effects of tubule occlusion agents on the permeability (Pashley, 1994). The equipment requires specialised equipment and experience. It often uses a dilution of plasma with phosphate buffered saline to simulate dentinal fluid. A micro-syringe is used to inject an air bubble into a micropipette, which is sealed between dentine samples and a pressurised buffer reservoir. If the apparatus is not sealed perfectly, the results will be affected. The position of the air bubble indicates the relative dentine permeability within each sample.

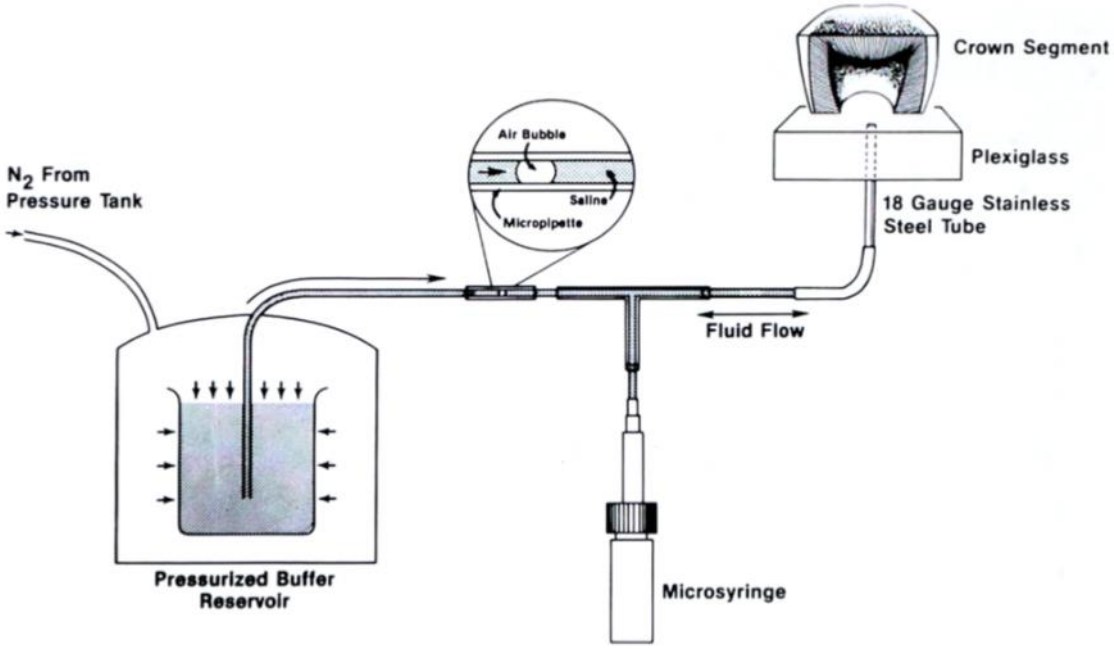


Figure 5 A device used to measure dentine permeability as hydraulic conductance from (Pashley *et al.*, 1988).

The permeability is affected by numerous factors including the size and anatomical variation of each dentine sample, which is affected by the formation of sclerotic dentine or reparative dentine (Pashley, 1986) and would need to be standardised. Variability in dentine specimens could therefore be a limitation and require that each dentine specimen act as its own control in studies investigating products used to occlude dentine tubules (Gregoire *et al.*, 2003). This is possible because the same area of each sample can be measured before and after treatment. However, a disadvantage is that due to the cross over effects of a treatment product, repeated investigations of the effect of tubule occluding agents on the same sample are not possible and would require that the test product first be removed (Ahmed *et al.*, 2005). Furthermore, despite hydraulic conductance providing a very useful quantitative technique for studying the effects of dentine occlusion products applied topically on dentine, surface imaging is also necessary to correlate surface deposits with any reduction in permeability (Pereira *et al.*, 2005).

1.21 Profilometry

Metrological systems may be used to measure three-dimensional (3D) surface texture and geometry. The hardware consists of either a contacting probe (stylus profilometers) or a non-contacting laser and sensor (optical profilometers). The use of profilometry is normally limited to air-dried samples. Stylus profilometers contact the surface of a sample to measure the surface topography, whereas optical profilometers use a beam of electromagnetic radiation. Stylus profilometers can exert high forces on a sample surface and have potential to damage the sample. The profilometer is also not useful at characterising the chemical and mechanical surface properties of a sample.

Data points are collected from the surface of the object of interest and analysed by software. Measurements include two-dimensional (2D) measurements (roughness, waviness, profile and 2D step-height) and three-dimensional (3D) measurements (surface area, step height and volume calculations) (Suga, 2007). In order to measure tooth wear using profilometry, it is important to measure a step between an unchanged reference area and experimental area that is subject to wear. This process has been used to measure changes in tooth wear taken from subsequent impressions *in vivo* (Bartlett *et al.*, 1997; Schlueter *et al.*, 2005) or *in vitro*, by protecting a designated area of enamel or dentine samples with acid resistant tape. In addition, surface-matching software is developed to superimpose sequential scans at different time intervals to detect changes (Chadwick *et al.*, 1997).

Profilometry is one accurate measurement of tooth wear in the laboratory for samples, which have undergone little or no preparation. By measuring surface topography, it can for example, provide an indication of the erosive potential of various acids (in addition to mineral loss) (Attin, 2006a). An *in vitro* study showed that when primary teeth were placed into various erosive beverages, the calcium concentration and titratable acidity of the beverages correlated with softening of dentine (Mahoney *et al.*, 2003).

1.22 Atomic Force Microscopy

Atomic Force Microscopy (AFM) is based on mapping of an atomic force field on the surface of an examined sample using a scanning probe. It offers the ability to analyse the surface properties of biomaterials non-destructively with nanometre level precision in ambient air or liquids. It consists of a tip, which is brought into light contact with the

surface under investigation and raster scanned across the surface by a piezoelectric scanner. According to the type of contact between the tip of the cantilever and the surface of the sample, it can be used in three modes; contact mode, non-contact mode and intermittent contact (tapping) mode. In the non-contact mode, the tip vibrates in a direction perpendicular to the surface, for examination of soft and elastic samples. In the tapping mode, the imaging probe is vertically oscillated at or near the resonant frequency of the cantilever. This is useful for biological samples, in order to eliminate the lateral forces on samples in contact mode, which could damage them. In all three modes, the distance the scanner moves vertically to maintain a constant deflection at every lateral data point is mapped in order to generate topographical images (Zapletalova *et al.*, 2004). The resolution depends on the material under investigation, but can provide a real topographical three-dimensional image of the sample surface with a resolution of 0.1 nm and lateral resolution of 1 nm. Compared to other metrological techniques such as stylus profilometry, the AFM offers better lateral resolution (Serry, 2011). It can also be used in real time and the AFM liquid scanner allows it to be used in liquid environments (Zapletalova *et al.*, 2004). The use of the cantilever tip can result in a number of difficulties and restrictions in measurement and sample preparation. When the tip scans across the surface of a sample, it introduces a dynamic interaction force between the tip and the surface, which is complicated and has been shown to make precise analysis of the sample difficult, and can influence the resolution of the surface image (Murayama and Omata, 2004). In addition, using AFM the final image is only a small proportion of the surface of the sample compared to other optical or electron imaging techniques and unsuitable for imaging large surface areas.

AFM has been used, together with SEM, to study individual dentine tubules and their occlusion using Nd: YAG laser irradiation. It was noted that a smooth sample surface is

preferable for the AFM (Kubinek *et al.*, 2007). It is increasingly being used for the surface nanocharacterisation of dentine and collagen and for the investigation of products and actives used in the management of DH (Sharma *et al.*, 2010). The equipment is a new and exciting area for research, but is limited not least because the cost and difficulty in operation might preclude its use to laboratories that specialise in AFM technologies (Wu *et al.*, 2004).

1.23 Models investigating dentine tubular occlusion

A model is defined as a 'simplified or idealized description or conception of a particular system, situation, or process...that is put forward as a basis for theoretical or empirical understanding, or for calculations, predictions, etc.' (Dictitionary, 2012). It may be used clinically or in the laboratory or both.

Physically blocking or occluding un-occluded dentine tubules and resisting acid challenge are the principle aims of treatment in DH (Markowitz and Pashley, 2008). Thus, a principle aim for investigations related to the aetiology and management of DH is to accurately measure dentine tubule occlusion. Clinical trials have often been conducted to measure DH, but results are variable due to subject-based reproducibility of DH (Ide *et al.*, 2001). Studies *in vivo* to measure the number of un-occluded dentine tubules are challenging. However, one method to replicate the clinical situation is to rely on *in situ* studies, which were developed from *in vitro* work, using images of the surface of dentine samples (Addy *et al.*, 2002). Using an *in situ* model, dentine samples can be mounted on appliances held in the oral cavity for several hours to subject them to the influence of intra-oral systems including the effect of saliva and influence of pellicle, and later studied using a surrogate approach using various types of laboratory equipment (Hooper *et al.*, 2005; West *et al.*, 1997). A standard approach to imaging the

dentine tubules has so far involved SEM imaging. To avoid a manual count of hundreds of images each containing numerous tubules, approaches evolved using visual semi-quantitative assessment of dentine tubule obstruction. A recent approach was developed *in situ on* 12 subjects to grade SEM images of impressions taken of the dentine surface using a visual ordinal scale and four examiners. It was designed to investigate treatments used to occlude dentine tubules (Barlow *et al.*, 2007). It has since been used *in vitro* to grade SEM images of the surface of dentine directly and non-invasively (Parkinson and Willson, 2011a). Other reviews suggest that a digital-image-based analysis would be a more sensitive and accurate way of quantifying tubule occlusion (Grenby, 1996). Studies have since attempted computational software for use as an image analysis tool to count the number and surface area of un-occluded tubules on SEM images. These include an *in situ* study (Banfield and Addy, 2004) and *in vitro* studies (Ahmed *et al.*, 2005; Ciocca *et al.*, 2007; Lee *et al.*, 2008).

There is currently no established method to measure dentine tubule occlusion. Studies investigating dentine tubule occlusion and tubular occlusion technologies *in situ* and in particular following acid challenge are lacking. Tools such as the visual ordinal scale have not been used as part of an *in situ* study to successfully illustrate dentine tubule occlusion directly on dentine and following an acid challenge. Other quantitative work using visual counts or software based analysis of the number of un-occluded or patent dentine tubules have not been compared to such a standard. SEM is a standard technique to measure dentine tubule occlusion, but to date no randomised control studies have been conducted *in situ* to measure dentine tubule occlusion using alternative imaging techniques such as ESEM or TSM. The latter may allow novel investigation of dentine samples *in situ* without additional sample processing, pre- and immediately post-treatment.

Aims of chapters 2, 3, 4 and 5

The aim of this research is to investigate clinically and in the laboratory the relationship between DH and tooth wear. The null hypothesis is that DH is not associated with tooth wear.

The aim of Chapter 2 was validation and calibration of the methods used to measure tooth wear and DH in this thesis. The null hypothesis was that the techniques are unable to measure DH and tooth wear.

The aim of Chapter 3 was to research the risk factors associated with the development and prevalence of tooth wear and DH in people aged 18- 35 years old seen in general dentistry in south east England. The null hypothesis is that there are no associations between risk factors, tooth wear and DH.

The aim of Chapter 4 was to create an *in situ* model to investigate dentine tubule occlusion of dentifrices following an agitated acid challenge. This was carried out using an established visual ordinal scoring method and SEM imaging. The null hypothesis is that the positive control dentifrices do not occlude the dentine tubules and are soluble in acid.

The aim of Chapter 5 was to compare a novel computerised and imaging method to measure dentine tubule occlusion with established techniques. This was carried out as part of a second *in situ* study to investigate dentifrices of different abrasivity designed to occlude dentine tubules and following an agitated acid challenge. The null hypothesis was that the automated computerised technique is unable to measure the

dentine tubules and is in poor agreement with the existing ‘standard’ (visual ordinal scale).

Chapter 2 Validation of methods

2.1 Section One: Validation of the Basic Erosive Wear Examination and Schiff index

2.1.1 Introduction

The Basic Erosive Wear Examination (BEWE), (section 1.15.1), is a screen for assessing the severity of tooth wear in general dental practice. It may also have uses for large research studies. For recording DH, the Schiff score was described in section 1.15.2. Both the BEWE and Schiff are based on a categorical ordinal scale 0-3. The BEWE uses a 6 sextant cumulative score per subject. For convenience in comparison to the BEWE, it is proposed that a sextant cumulative score will also be used for recording Schiff per subject.

The BEWE (and Schiff) recorded, as a sextant score per subject, have not been previously compared with a score taken from all tooth surfaces per subject. This would help validate if the sextant score provide an adequate representation of the overall experience of tooth wear and DH. Therefore, this section describes the validation of the BEWE and Schiff scores used later in Chapter 3. The aim is to investigate if the Schiff and BEWE sextant scores are adequate tools on which to compare risk factors, tooth wear and DH per subject, in Chapter 3 of this thesis. Further aims were to investigate if the BEWE sextant cumulative score and Schiff sextant cumulative score provide an accurate representation of the wear and DH recorded on all tooth surfaces per subject. This study was carried out between June 2011 and February 2012. The null hypothesis was that the BEWE and Schiff sextant scores do not reflect the tooth wear and DH occurring on all tooth surfaces.

2.1.2 Method

2.1.2.1 Training and calibration for BEWE

The author was trained and calibrated on the use of BEWE by recording a score for each of 90 images of tooth surfaces and repeated by an expert 'gold standard' examiner. In order to assess the agreement of BEWE scores between the examiner and the gold standard, the scores were cross tabulated. An intra-examiner Cohen Kappa value (k) was then obtained. Operator re-training and calibration took place at various time points during the study and the k was $\geq$0.7 as shown in Table 8.

Table 8 Intra-examiner reliability

Time period	Intra-examiner k
June 2012	0.732
September 2012	0.839
December 2012	0.854
February 2012	0.870

2.1.2.2 Training and calibration for Schiff

This took place during examination of three subjects by both examiners and Schiff was recorded on every tooth surface. The scores were cross tabulated and an intra-examiner Cohen Kappa value (k) calculated to give >0.8. This exercise was repeated during the study.

2.1.2.3 Sample size

The sample size is described in Chapter 3.

2.1.2.4 Ethical approval

The study received ethical approval (11/H0801/3). All subjects were provided with patient information sheets and had an opportunity to ask questions and were required to provide written consent prior to enrolling in the study. The patient information sheet and consent forms are available on request.

2.1.2.5 Inclusion and exclusion criteria

Participants were recruited who conformed to the inclusion and exclusion criteria listed below and who consented to the study.

Inclusion criteria included;

- Subjects of either gender, ambulatory, who were attended for a routine dental appointment in hospital or dental practice,
- Aged 18–35 years, inclusive,
- Having a good understanding of the English language,
- Understands and is willing, able and likely to comply with all study procedures and restrictions,
- Good health (in the opinion of the examiner) without clinical abnormality or abnormal medical history.

Exclusion criteria included;

- Subjects incapable of responding to the questions,
- Subjects having oral pathology – haemophilia, using anti-coagulants (including plaque anti-aggregates),
- Subjects currently using fixed maxillary or mandibular orthodontic appliances,
- Subjects who had used pain relieving drugs or had used a topical analgesic in the preceding 24 hours,

- Subjects who required antibiotic cover,
- An employee of the study site or their immediate family member.

2.1.2.6 General procedure

The teeth were dried using compressed air and examined without magnification under normal dental surgery conditions with good lighting. Buccal/bucco-cervical, occlusal/incisal and lingual/palatal of all tooth surfaces were examined. Missing teeth, restored surfaces, traumatised or carious teeth and third molars were excluded.

Subjects were recruited from general practice and hospital university locations. Of necessity the sampling was by a convenient method with all possible participants offered recruitment on the sessions attended by the principle examiner. If subjects conformed to the inclusion and exclusion criteria they were offered the option of participating. Those who accepted were examined. Further details are given in Chapter 3.

2.1.2.7 Assessment of tooth wear

BEWE scores were recorded on each tooth surface by the same examiner. Based on the guidelines for recording BEWE (Bartlett *et al.*, 2008), tooth surfaces were scored using an ordinal scale 0-3;

- 0= No wear,
- 1= Initial loss of surface texture,
- 2= Hard tissue loss <50% of the surface area,
- 3= Hard tissue loss $\geq$50%.

Figure 6 shows the BEWE scores for buccal, occlusal/incisal and lingual/palatal tooth surfaces, which would be 0, 3 and 1 respectively.

Figure 6 Tooth surfaces

The data were collected for the whole group and each subject had main data outcomes;

- BEWE sextant cumulative score per subject,
- Percentage of all tooth surfaces on each subject with a BEWE score of 1, 1 and above, 2, 2 and above and 3,
- Percentage of buccal, occlusal/incisal and lingual/palatal tooth surfaces on each subject with a BEWE score of 1, 1 and above, 2, 2 and above and 3,
- Highest BEWE score per subject,
- Highest BEWE score per tooth surface (buccal, occlusal/incisal and lingual/palatal) per subject.

In order to calculate the BEWE sextant cumulative score or risk score, the sum of the highest score from each oral sextant was calculated. An example is shown in Figure 7.

2	0	1
2	1	2

Figure 7 BEWE calculated as a sextant cumulative score

The BEWE sextant cumulative score provides an assessment of the subject's overall risk of tooth wear and the 'medium' and 'high' categories may involve operative management (Bartlett *et al.*, 2008). The risk categories are none (≤2), low (3-8), medium (9-13) or high (≥14).

A cumulative percentage score was also calculated as the proportion of all tooth surfaces which had a BEWE score 1, 1 and above, 2 and above and 3 for each subject in the study. An example for buccal tooth surfaces is shown in Figure 8. This shows 28 buccal tooth surfaces examined. For this subjects buccal surfaces, a BEWE score 0 was 39.3% (n=11), score 1 was 46.4% (n=13), score 1 and above was 60.7% (n=17), 2 and above was 14.3% (n=4) and score 3 was 0% (n= 0).

Maxilla	1	1	2	1	0	0	0	0	0	0	0	0	0	1
Mandibular	1	2	1	1	0	1	1	0	1	1	2	1	2	1

Figure 8 BEWE scores for buccal tooth surfaces on a subject

The highest BEWE score was also recorded for each subject per tooth surface (buccal, occlusal/incisal and lingual/palatal).

2.1.2.8 Assessment of DH

DH was assessed using an evaporative stimulus as described in section 1.15.2. Using Schiff, air was directed from a three in one tip of a dental syringe at ninety degrees to

the tooth surface from a distance of approximately one cm for one second (Pashley, 1990c; Schiff *et al.*, 1994) as described in clinical studies (Mason *et al.*, 2010). The adjacent tooth surfaces were shielded using a gloved finger. The response to DH was recorded on every tooth surface using the Schiff index. This is an ordinal scale recorded as follows;

0= Subject does not respond to air stimulus,

1= Subject responds to air stimulus but does not request discontinuation of stimulus,

2= Subject responds to air stimulus and requests discontinuation or moves from stimulus,

3= Subject responds to the air stimulus, considers stimulus to be painful and requests discontinuation of the stimulus.

The procedure was then repeated on each subject using a second tool designed to record the presence or absence of DH on each tooth surface based on subject feedback. This index will be referred to as the DH index. This is a binary scale recorded as follows;

0= No DH,

1= DH present.

In similarity to the assessment of tooth wear, a sextant cumulative score was calculated for Schiff on each subject. In the same way as the BEWE sextant cumulative score, this involved summation of the highest Schiff score recorded on a tooth surface in each oral sextant. Then, a cumulative percentage score was also calculated as a proportion of all tooth surfaces (buccal, occlusal/incisal and lingual/palatal) for Schiff 1, 1 and above, 2, and 2 and above, and 3 per subject. The highest Schiff score was also obtained per subject for all tooth surfaces (buccal, occlusal/incisal and lingual/palatal).

2.1.2.9 Statistics

In order to assess intra-examiner reproducibility, examinations were repeated a second time on every tenth patient who was recruited. This second examination occurred immediately after the first for convenience and the BEWE, Schiff and DH scores were recorded again for every tooth surface. Then, sextant cumulative scores, cumulative percentage score per tooth surfaces, and highest scores per tooth surface were recorded for these subjects.

The agreement between the quantitative data sets from the first and second clinical examinations were assessed using intra-class correlation coefficients. The overall data was then analysed descriptively.

Spearman correlation coefficients (and p values) were used to assess if there was a relationship between sextant cumulative scores per subject and the cumulative percentages (derived from all tooth surfaces) per subject or highest scores per subject. This analysis was performed on the data from the whole group, for the BEWE and Schiff data respectively.

2.1.3 Results

The demographics and further details of prevalence of tooth wear and DH of the population are included in Chapter 3.

2.1.3.1 Reproducibility

Intra-examiner reproducibility of repeated clinical outcomes taken from tooth surfaces on 10% of subjects showed intra-class correlation coefficients $\geq$0.96.

2.1.3.2 Summary of BEWE sextant cumulative, BEWE per tooth surface and BEWE highest per subject

The BEWE sextant cumulative or risk score per subject ranged from 0 to 16 (median 7, inter-quartile range (IQR) 5-9, mean 6.5, standard deviation 3.77). At the subject level, the highest BEWE score recorded on at least one tooth surface of 1 was 44% (n=153), of 2 was 37% (n=129), of 3 was 10% (n=36) and of 0 was 9% (n=32).

Figure 9 shows the percentage distribution of BEWE scores per tooth surface and as a highest per subject. Tooth surfaces had a BEWE 0 (40%, n=9, 716), BEWE 1 (36%, n=8, 673), BEWE 2 (20%, n=4, 741) or BEWE 3 (4%, n=883). This is based on the whole group data.

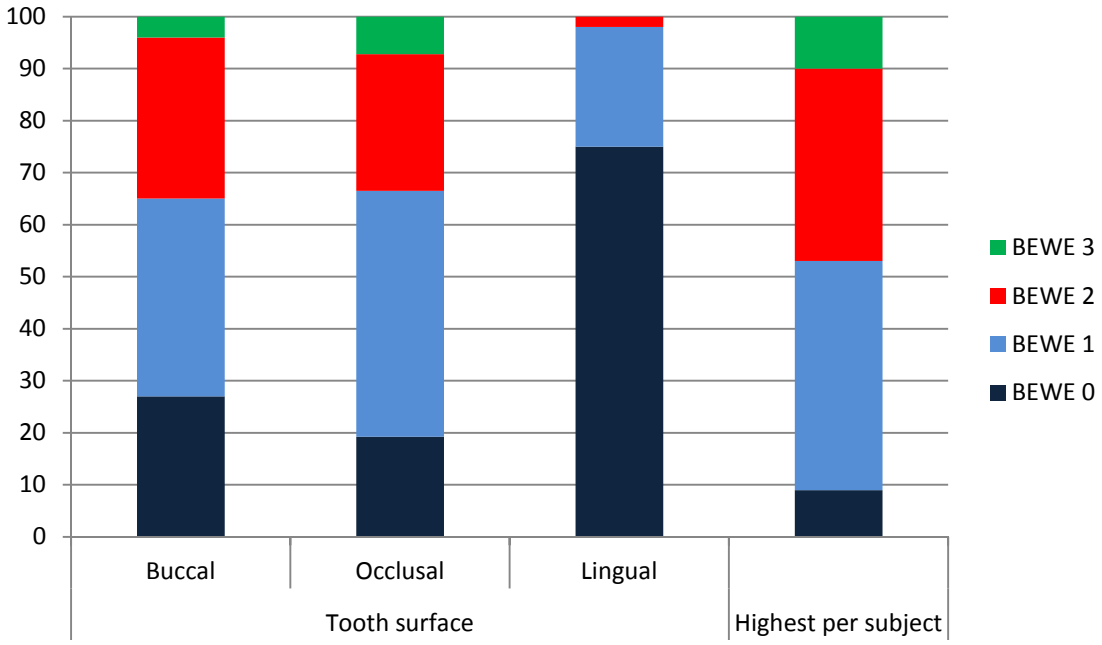


Figure 9 Percentage distribution of BEWE scores per tooth surface and as a highest per subject

The median (IQR) of cumulative percentage BEWE scores on buccal, occlusal and lingual surfaces is shown in Table 9. These medians were analysed from the whole

group data. The median percentage for a BEWE score of 1 and above was 43% (IQR 21-57) on occlusal surfaces, 17% (IQR 0-27) on buccal surfaces and 0% (IQR 0-8) on lingual surfaces.

Table 9 Median (inter-quartile range) of cumulative percentage BEWE per tooth surface

Cumulative BEWE percentages	**Tooth surface**			**Total**
	Buccal	**Occlusal**	**Lingual**	
BEWE (1)	9 (0-19)	29 (19-42)	0 (0-8)	15 (9-21)
BEWE (1) and above	17 (0-27)	43 (21-57)	0 (0-8)	19 (12-29)
BEWE (2)	12 (0-19)	28(4-39)	0 (0-0)	8 (3-12)
BEWE (2) and above	0 (0-8)	8 (0-17)	0 (0-0)	3 (0-9)
BEWE (3)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)

For those subjects with a highest BEWE score of 1 on at least one tooth surface, the BEWE sextant cumulative score was always less than or equal to 8 ('low' risk category). Amongst those subjects with a highest BEWE score of 3 on at least one tooth surface (10%, n=35), the BEWE sextant cumulative score ranged from 9 to 16 ('medium' to 'high' risk categories); except on one subject who had sextant score of 4 ('low' risk).

2.1.3.3 Correlation of BEWE sextant cumulative and BEWE per tooth surface

Table 10 shows the Spearman correlation coefficients (95% confidence intervals) and p values for the relationship between BEWE sextant cumulative score (subject based) and the BEWE percentage cumulative score (subject based) for all tooth surfaces examined. This analysis was based on the whole group data.

Correlations for tooth surfaces with a BEWE 1 and above and a BEWE 2 and above were >0.8 ($p<0.001$), BEWE 1 only were 0.528 ($p<0.001$) and BEWE 3 only were 0.513 ($p<0.001$). Amongst scores 1 and above, 2 and 2 and above, the lingual surfaces had less correlation (≤ 0.3) than the buccal and occlusal surfaces (≥ 0.7). There was no recorded BEWE 3 on lingual tooth surfaces and hence this could not be correlated to the BEWE sextant cumulative score.

Table 10 Spearman correlation coefficients (95% confidence intervals) and p values for relationship between 'BEWE sextant cumulative' score and cumulative BEWE percentages per subject, for each tooth surface

Cumulative BEWE percentages	**Tooth surface**			**Total**
	Buccal	**Occlusal**	**Lingual**	
BEWE (1)	0.457* [0.370-0.536]	0.356* [0.260-0.444]	0.293* [0.194-0.386]	0.528* [0.448-0.600]
BEWE (1) and above	0.696* [0.638-0.746]	0.788* [0.744-0.825]	0.157** [0.057-0.257]	0.853* [0.821-0.879]
BEWE (2)	0.685* [0.601-0.743]	0.732* [0.687-0.798]	0.245* [0.189-0.287]	0.829* [0.771-0.876]
BEWE (2) and above	0.674* [0.613-0.728]	0.676* [0.615-0.730]	0.333* [0.237-0.423]	0.805* [0.762-0.837]
BEWE (3)	0.307* [0.209-0.399]	0.455* [0.367-0.534]	¶	0.513* [0.431-0.586]

¶ All scores zero

*p<0.001, **p=0.003

Table 11 shows the Spearman correlation coefficients (95% confidence intervals) and p values for the relationship between BEWE sextant cumulative score (subject based) and the BEWE highest per subject score. This analysis is based on the whole group data. The correlation for the highest BEWE scores recorded on all tooth surfaces was >0.7 ($p<0.001$), for buccal and occlusal tooth surfaces was >0.7 ($p<0.001$) and for lingual tooth surfaces was 0.4 ($p<0.001$).

Table 11 Spearman correlation coefficients (p values) for relationship between 'BEWE sextant cumulative' and BEWE highest per subject

Tooth surface			Total
Buccal	**Occlusal**	**Lingual**	
0.750* [0.700-0.793]	0.699* [0.642-0.749]	0.406* [0.315-0.490]	0.785* [0.741-0.822]

*p<0.001

2.1.3.4 Summary of Schiff sextant cumulative, Schiff per tooth surface and Schiff highest per subject

The Schiff sextant cumulative or risk score per subject ranged from 0 to 13 (median 7, inter-quartile range (IQR) 5-9, mean 2.16, standard deviation 2.90). At the subject level, the highest Schiff score recorded on at least one tooth surface of 0 was 42% (n=160), of 1 was 30% (n=105), of 2 was 17% (n=58) and of 3 was 8% (n=27).

Figure 10 shows the percentage distribution of Schiff scores per tooth surface and as a highest per subject. Tooth surfaces had a Schiff 0 (68%, n=16, 383), Schiff 1 (21%, n=5, 060), Schiff 2 (8%, n=1, 927) or Schiff 3 (3%, n=723). This is based on the whole group data.

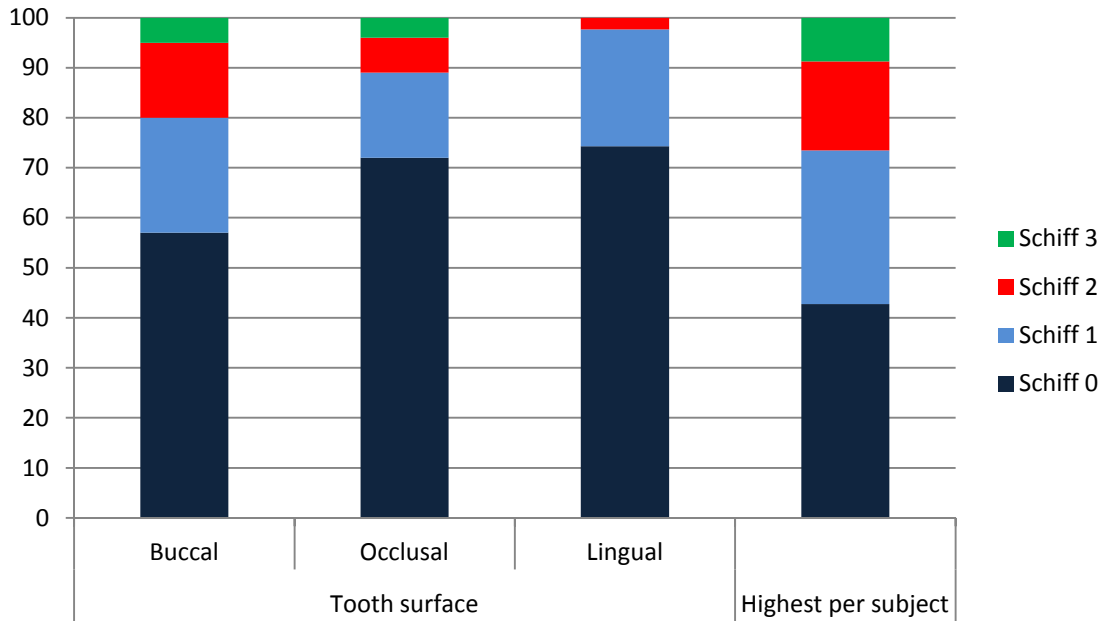


Figure 10 Percentage distribution of Schiff scores per tooth surface and as a highest per subject

The median (IQR) of cumulative percentage Schiff scores on buccal, occlusal and lingual surfaces is shown in Table 12. These medians were analysed from the whole group data. The median percentage for a BEWE score of 0 and above was 0% (IQR 0-0) all tooth surfaces.

Table 12 Median (inter-quartile range) of cumulative Schiff percentages per tooth surface.

Cumulative Schiff Percentages	**Tooth surface**			
	Buccal	**Occlusal**	**Lingual**	**Total**
Schiff (1)	0 (0-8)	0 (0-0)	0 (0-0)	1 (0-3)
Schiff (1) and above	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Schiff (2)	0 (0-0)	0 (0-0)	0 (0-0)	1 (0-5)
Schiff (2) and above	0 (0-0)	0 (0-0)	0 (0-0)	1 (0-5)
Schiff (3)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)

2.1.3.5 Correlation of Schiff sextant cumulative and Schiff per tooth surface

Table 13 shows the Spearman correlation coefficients (95% confidence intervals) and p values for the relationship between the Schiff sextant cumulative score (subject based) and the Schiff percentage cumulative score (subject based) for all tooth surfaces examined. This analysis was based on the whole group data. Correlations for tooth surfaces with a Schiff 1, Schiff 1 and above, Schiff 2 and a Schiff 2 and above were >0.7 ($p<0.001$), Schiff 3 only were 0.449 ($p<0.001$). Correlations for buccal surfaces were higher than occlusal surfaces, which in turn were higher than lingual surfaces. There was no recorded Schiff 3 on lingual tooth surfaces and hence this could not be correlated to the Schiff sextant cumulative score.

Table 13 Spearman correlation coefficients (95% confidence intervals) and p values for relationship between 'Schiff sextant cumulative' score and cumulative Schiff percentages per subject, for each tooth surface

Cumulative Schiff percentages	**Tooth surfaces**			**Total**
	Buccal	Occlusal	Lingual	
Schiff (1)	0.808* [0.750-0.843]	0.566* [0.493-0.632]	0.554* [0.499-0.612]	0.898* [0.821-0.967]
Schiff (1) and above	0.886* [0.831-0.924]	0.669* [0.601-0.743]	0.590* [0.503-0.687]	0.982* [0.932-1.040]
Schiff (2)	0.648* [0.599-0.704]	0.460* [0.408-0.517]	0.219* [0.179-0.285]	0.720* [0.673-0.775]
Schiff (2) and above	0.692* [0.589-0.743]	0.512* [0.478-0.564]	0.219* [0.189-0.265]	0.757* [0.701-0.798]
Schiff (3)	0.328* [0.287-0.267]	0.326* [0.296-0.356]	¶	0.449* [0.401-0.511]

¶ All scores zero

*p<0.001

Table 14 shows the Spearman correlation coefficients (95% confidence intervals) and p values for the relationship between Schiff sextant cumulative score and the Schiff highest per subject score. The correlation for the highest Schiff scores recorded on all tooth surfaces was >0.9 (p<0.001), for buccal tooth surfaces was >0.8 (p<0.001), for occlusal was >0.6 and for lingual tooth surfaces was >0.5 (p<0.001).

Table 14 Spearman correlation coefficients (95% confidence intervals) and p values for relationship between 'SCHIFF sextant cumulative' and SCHIFF highest per subject

Tooth surface			**Total**
Buccal	Occlusal	Lingual	
0.874* [0.812-0.931]	0.667* [0.602-0.718]	0.576* [0.489-0.638]	0.963* [0.920-0.989]

*p<0.001

2.1.4 Discussion

This is the first study to the author's knowledge that has compared the total BEWE and Schiff scores to individual tooth surfaces and shown a relationship. For BEWE, it validates the use of the sextant scores particularly between scores of 1 and above, 2 and 2 and above, but not so much at score 1 and 3. For Schiff, it validates the use of the sextant scores particularly between scores of 1, 1 and above, 2 and 2 and above, but not so much at score 3. The sextant scores also correlate well to the highest BEWE or Schiff scores recorded per subject respectively.

This study shows that the use of a sextant score for BEWE and Schiff reflect the tooth wear and DH occurring as a percentage of tooth surfaces per subject or as a highest score recorded per subject. The sextant score does not reflect advanced wear or DH (scored as 3), but this is probably a reflection of the low prevalence of these scores in

this study. In general, the study has shown that the BEWE and Schiff sextant scores are useful screening tools for assessing tooth wear and DH respectively and may avoid the need for recording tooth wear and DH on every tooth surface.

2.1.4.1 BEWE sextant cumulative score

The BEWE scoring system, in contrast to other tooth wear scoring systems, does not distinguish enamel loss and dentine exposed (Bartlett *et al.*, 2008; Ganss *et al.*, 2006). Previous studies divulge that it can be particularly difficult to differentiate lesions localised to enamel or dentine especially in the cervical area of teeth and that this could lead to diagnostic uncertainties (Holbrook and Ganss, 2008). Severe wear has also been shown to be masked by restorations in the cervical area (Donachie and Walls, 1995). However, this study demonstrates that the BEWE sextant cumulative score relates to tooth wear scores from all tooth surfaces. In particular, correlation of the BEWE sextant cumulative to the BEWE percentage scores for tooth surfaces per subject and to the highest BEWE score per subject, were greater for buccal and occlusal surfaces than for lingual tooth surfaces. Unlike the latter, buccal and occlusal tooth surfaces were shown to more likely have dentine exposure and these surfaces included buccal NCCLs. Despite this, the study also showed that percentage scores on lingual tooth surfaces did not correlate well with the BEWE sextant cumulative score. This may be explained by the median percentage BEWE for lingual surfaces, which was 0 (IQR 0-0). Lingual tooth wear, when present (25%, n=87 subjects), was often localised to enamel. Other clinical studies similarly recorded less wear on lingual (Khan *et al.*, 1999; Radentz *et al.*, 1976), compared to buccal tooth surfaces, but fortunately treatment would not be required in these cases. The BEWE sextant cumulative score identifies tooth wear, which in this study was more likely on buccal or occlusal tooth surfaces.

The BEWE sextant cumulative score relates more to tooth surfaces with a BEWE score of 1 and above, 2 and 2 and above ($\geq$0.8, $p<0.001$) and to a slightly lesser extent BEWE scores 1 and 3 ($\leq$0.5, $p\leq0.001$). This shows that the BEWE sextant cumulative score relates well to tooth surfaces with early wear and distinct tissue loss involving dentine, but less to those subjects with minimal wear affecting enamel (BEWE score 1 only) or extensive wear affecting at least 50% of a tooth surface (BEWE score 3 only). Although this might suggest that subjects who have minimal or advanced wear may not be placed into the appropriate BEWE risk categories for tooth wear, the risk categories for BEWE sextant cumulative; as described by Bartlett *et al.* 2008; include a variation in the BEWE sextant cumulative score, which avoids over- or under-estimating the amount of tooth wear. For example, subjects who had a highest BEWE score on any tooth surface of 1 had a sextant cumulative score not greater than 8 and hence, the risk score for these subjects would still be 'low'. In addition, among subjects who had a BEWE 3 on a tooth surface (4%, n=14), the risk score was 'medium' or 'high' in all but one subject. Hence there could be potential for reduction in the accuracy of BEWE in rare cases of advanced localised wear. Overall however, correlation of the BEWE sextant cumulative to the highest BEWE score recorded on a tooth surface per subject was greater than 0.7 ($p<0.001$).

Since this study was conducted, another study has compared the BEWE to another index (TWI), on 164 adult patients. This demonstrated that the BEWE scores showed a similar distribution to the TWI scores. However, the inter- and intra-examiner reproducibility were moderate (weighted Kappa values=0.43 and 0.57 respectively) (Dixon *et al.* 2012).

The BEWE was adopted based on the Basic Periodontal Examination (BPE) or Community Index of Periodontal Need (CPITN) (Ainamo *et al.*, 1982). CPITN is now

commonly used in dental practice to screen periodontal disease by measuring the maximum pocket depth in each oral sextant using a probe and adds these together to create a sextant score. This provides an indication of the patient's risk of periodontal disease. Previous research has nonetheless shown that the CPITN may under- or over-estimate the level of periodontal pocketing or fail to reflect the level of pocketing in all teeth. Similar to this study, one paper compared the CPITN to a measurement of periodontal probing taken from every tooth, but it found that the CPITN fails to measure periodontal disease in comparison to the full mouth assessment ($p<0.001$) (Bassani *et al.*, 2006). Another study also reports that CPITN often under-estimates the depth of periodontal pocketing in sextants that have deeper pockets (Diamanti-Kipioti *et al.*, 1993) despite the CPITN formerly being reported as having better suitability for severe disease diagnosis (Ainamo and Ainamo, 1985). The problems with over-estimating the depth of periodontal pocketing using CPITN may be due to the use of a periodontal probe to measure pocket depth, but the CPITN itself is also a partial score, with one score recorded per sextant and can also under-represent the disease. Similarly, the BEWE index might also be expected to under-estimate the level of tooth wear due to its sextant design and this was the case in one subject in our study. However, the BEWE is unlike previous commonly used tooth wear indices such as the Smith and Knight, which were more detailed and could over-estimate the amount of tooth wear by asking the examiner to estimate the proportion of teeth affected by tooth wear (Ganss *et al.*, 2006). In addition, the study demonstrates that the BEWE sextant cumulative score can be a useful screening tool that does reflect the total amount of wear occurring in the mouth overall.

2.1.4.2 Schiff sextant cumulative score

The Schiff sextant cumulative score relates more to tooth surfaces with Schiff score of 1, 1 and above, 2 and 2 and above (≥ 0.8, $p<0.001$) and to a slightly lesser extent Schiff

scores of 3 (≤0.5, p<0.001). It should be noted that only 5% (n=15) of buccal tooth surfaces and 4% (n=14) of occlusal surfaces had a Schiff score of 3. In addition, there was no score of 3 on lingual tooth surfaces. Schiff score of 3 involves subjects who respond to the air stimulus, consider the stimulus to be painful and request discontinuation of the stimulus. Good differential diagnosis is required on behalf of the clinician in ensuring this pain is due to DH and not due to other conditions, which may cause pulpal pain (Addy, 2002). The Schiff sextant cumulative score correlates more to buccal surfaces than occlusal and lingual surfaces, which had less recorded DH.

Using the Schiff as a sextant cumulative score might underestimate the level of DH in rare circumstances, in similarity to the BEWE sextant score, as described in section 2.1.4.1.

Comparison of DH, tooth wear and aetiologies from this study will be described in detail in Chapter 3.

2.1.4.3 Reproducibility

The reproducibility of clinical assessment on 10% of the sample was high in this study, but this may be explained in part because subjects were examined a second time within 30 minutes of the first exam. This was to avoid inconvenience to the subject and the practice in recalling the same patient at another appointment. However, there would ideally be a time lapse between the first and second clinical examination. A convenience sample was also used as this study took place at various sites and aimed to accommodate all subjects willing to complete the study and who fulfilled the necessary inclusion/exclusion criteria. A second method to ensure consistency using the BEWE and Schiff scoring systems in this study was to use a single examiner throughout the study who received on-going training. Intra-examiner agreement to an expert examiner through calibration exercises remained at >0.7 throughout the study

for BEWE. Although a single examiner was used in this study, other studies have also demonstrated good agreement between various examiners for the BEWE sextant cumulative score (>0.7) (Smith and Knight, 1984a) and this would suggest extrapolation of the results to other studies is possible for use in wider epidemiological research.

2.1.4.4 Conclusion

This study has validated the BEWE sextant cumulative score and a novel Schiff sextant cumulative score. For the purposes of this PhD, it shows that both these scoring methods provide an accurate representation of the tooth wear and DH processes occurring on all tooth surfaces. In addition, the use of a novel Schiff sextant cumulative score will help comparison of DH to the tooth wear.

In tooth wear, as in DH, there is no gold standard for clinical evaluation. There is a need for such a standard given the importance of these disease processes. The clinical implications for the BEWE sextant cumulative score is that it provides a simple method to alert clinicians to the tooth wear process. It records wear more consistently than previous commonly used indices, which were more complicated and is useful in selecting those patients who may require treatment management. Bearing in mind all the detailed analysis the main aim of the BEWE is to assist GDPs in screening for erosive tooth wear. The findings from this study lend support to its use and show that it is fit for purpose.

2.2 Section two: Establishment of dentine discs for the *in situ* study of tubule occlusion

2.2.1 Introduction

Chapter four and five of this thesis describe *in situ* studies used to investigate dentine tubule occlusion. This involved using samples or discs of dentine derived from the coronal section of root dentine, as described in the section 1.16. The aim is to create samples of dentine with patent or un-occluded dentine tubules, which are ideally perpendicular to the test surface of the dentine.

2.2.2 Method

2.2.2.1 Teeth

Teeth for the dentine samples were sought from patients attending for an assessment appointment at the Oral Surgery Department at King's College Hospital NHS Foundation Trust, prior to undergoing routine extractions of their third molars (King's College Hospital NHS Foundation Trust Research Ethics Committee Approval reference 09/H0808/109). All clinicians who were identifying potential subjects had been provided with information and training by the research team. This was to ensure that the clinicians could identify suitable subjects (according to the principle inclusion and exclusion criteria for recruitment of participants to donate extracted teeth for the research study). It was also to ensure that the clinicians were familiar with the proposed research work so that they would be able to answer any questions that subjects may have at that stage.

Once consent had been obtained the teeth were then extracted following normal procedures. The extracted teeth were collected by the research team and anonymised (i.e. there was to be no link to the patient because samples were anonymised after collection and no personal data were to be collected or stored). Following collection, the teeth were placed for 1 hour in a 5% sodium hypochlorite solution (20, 000ppm available chlorine) before being placed in a de-ionized water container for transfer to the Department of Biomaterials located on Floor 17 of Guy's Tower, King's College London Dental Institute, Guy's and St. Thomas' NHS Foundation Trust, London Bridge, SE1 9RT.

The principle inclusion criteria included:

- Adults aged 18-65,
- Patients who needed teeth extracted for clinical reasons,
- Teeth free from dental caries,
- Patients able to provide written informed consent.

The principle exclusion criteria included:

- Patients aged<18 years or >65 years old.

2.2.2.2 Preparation of dentine samples

The crucial stages of sample forming involve:

- Sectioning,
- Polishing,
- Processing.

The sectioning process is important to obtain dentine samples for study using laboratory or *in situ* methodologies. Polished samples are important to reduce anatomical variation on the surface of each sample and to detect changes due to

erosion and abrasion, which are very small (Attin, 2006b) and may otherwise not be detected using surface imaging for example using TSM.

Teeth were sectioned at the cement-enamel junction (CEJ) and again 2mm apical from the CEJ using a diamond wafer blade (XL 12205, Benetec Ltd, London, UK). These discs were sectioned to reveal 5mm x3mm x2mm pieces of root dentine. Approximately 3-4 pieces were created from each tooth. The sectioning procedures are shown in Figure 11.

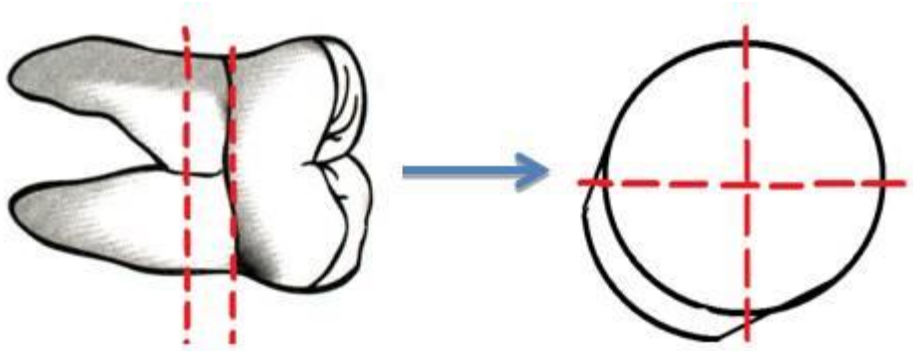


Figure 11 Sectioning of tooth below the cement-enamel junction to produce dentine samples

DH has been reported frequently at the cervical region of teeth. A second reason for using dentine from the cervical region was because at these regions, the dentine tubules radiate parallel from the pulp to the surface of the tooth (Mjor and Nordahl, 1996). This helped ensure dentine tubules were cross-sectioned as close to 90° to the surface of each sample as possible. Samples were placed in polyurethane vacuum packed moulds 6mm x 8mm x 2mm (width, length and depth respectively) filled with a bis-acryl composite material (Protemp4, 3M ESPE, Seefeld, Germany) and cured (Figure 12). The dentine nearest the pulp was embedded first with the sample at 90° to the mould. This was to ensure that the dentine tubules in the middle of each sample

were at 90° to the surface of the sample. Once cured, the samples were removed from the polyurethane moulds.

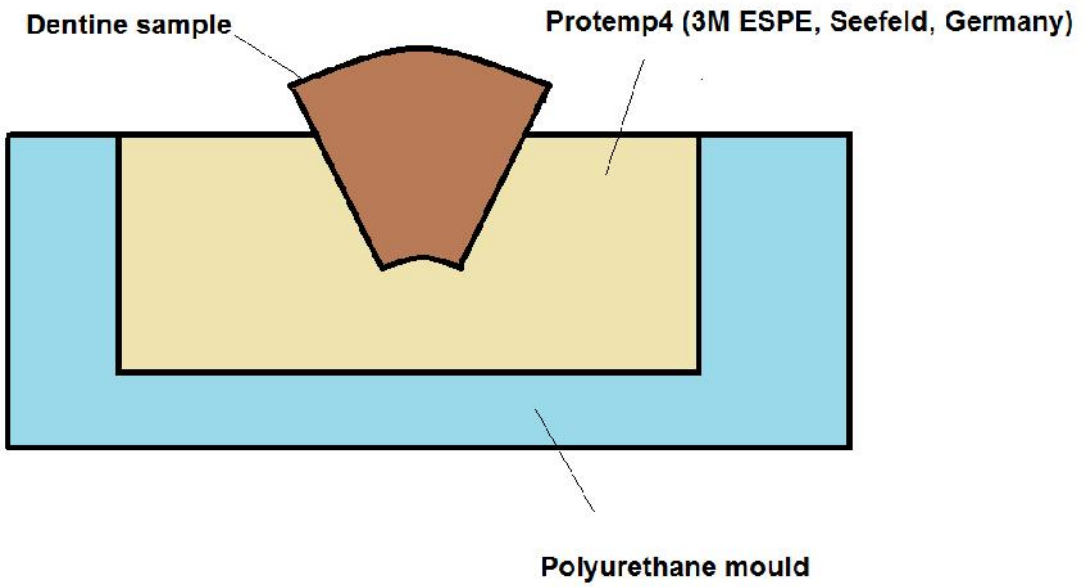


Figure 12 Embedding of dentine sample

The surface of samples were polished, under copious water irrigation, in jigs made from bis-acryl composite (Protemp4, 3M ESPE, Seefeld, Germany) held consistently face down by a semi-automated polishing head with 10 N applied force (Vector LC Power Head, Buehler, Lake Bluff, Illinois, USA) to a polishing disc rotating in the opposite direction attached to a water-cooled rotating polishing machine (Meta-Serv 3000 Grinder-Polisher, Buehler, Lake Bluff, Illinois, USA). The samples were sequentially polished consecutively using 80, 500, 1, 200 and 2, 400 grit silica carbide discs (Versocit, Struers A/S, Copenhagen, Denmark) to produce smooth, flat areas of dentine with approximately 1mm of the surface of the samples removed by grinding and polishing. The grinding procedure was necessary to remove the excess Protemp adjacent the dentine and used the 80 and 500 grit silica carbide discs. The polishing procedure was then used to remove 200μm from the surface of the dentine using the 1,

200 and 2, 400 grit silica carbide discs. This was measured using micrometer callipers on all samples. The reverse and sides of each sample were also polished to create parallel surfaces.

Samples were disinfected in sodium hypochlorite (20, 000ppm), washed in copious distilled water and then etched in 6% citric acid for 1 minute under gentle agitation (Stuart Scientific, Mini Orbital Shaker, SO5) to expose the dentine tubules. Figure 13 shows the effect on appearance of the sample using TSM with and without the agitation. The sample with agitation has more un-occluded dentine tubules visible, of a greater diameter.

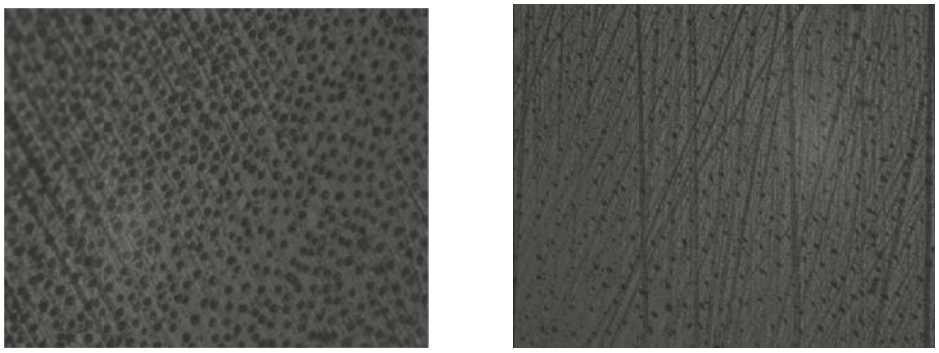


Figure 13 TSM (x40/0.55 NA) dry lens image of a dentine sample etched in 6% citric acid using gentle agitation (left) and no agitation (right)

Samples were stored in 0.9% Sodium Chloride until used. This length of time did not exceed 2 weeks.

Tandem scanning confocal microscopy (TSM) (Noran Instruments, Middleton, WI, USA) in conjunction with a M-Plan 20x SLWD Brightfield Objective (x20/0.35 NA objective) were used to screen all samples prior to their use in order to confirm sample integrity and establish tubule density and orientation visually prior to use. Each dentine sample was identified with a unique number on the reverse of the dentine sample using a permanent marker.

2.2.2.3 Embedding material

Ten samples were prepared to assess two different embedding materials. One approach involved mounting dentine in acrylic. However, SEM images taken of these samples suggested penetration of acrylic into the dentine tubules and across the surface of the dentine sample once polished, unlike a “Protemp” material. This is shown in Figure 14. Following this early validation work “Protemp” (3M ESPE, Seefeld, Germany) was used to mount the dentine because it had higher filler content and showed none of the problems exhibited by acrylic. Figure 15 shows TSM images taken from a sample mounted in Protemp (3M ESPE, Seefeld, Germany), using an oil-based objective lens (x40/0.55 NA) in order to visualise dentine tubules underneath the smear layer and possible penetration of the embedding material into the tubules. The bright lines represent reflection of light from within the dentine tubules, which are empty. The reflectance disappears at the base of the sample, which represents dentine closer to the pulp and which is adjacent to the embedding material.

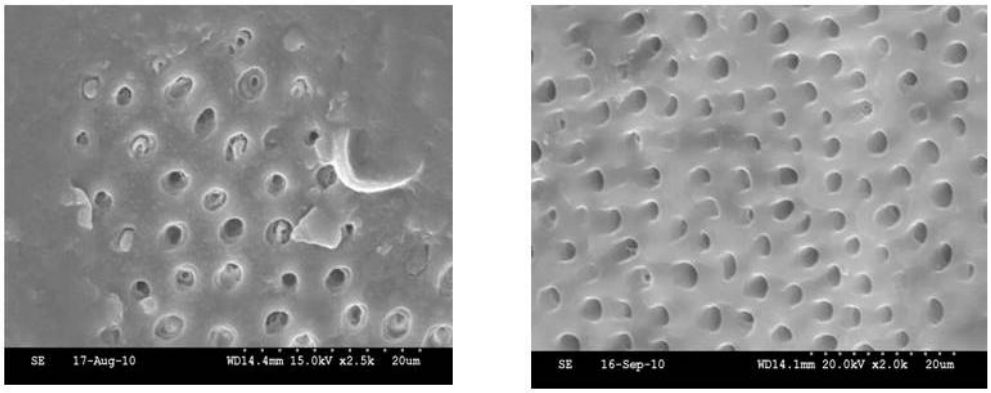


Figure 14 SEM images (x2000) of dentine mounted in acrylic resin (left) or Protemp4 (right)

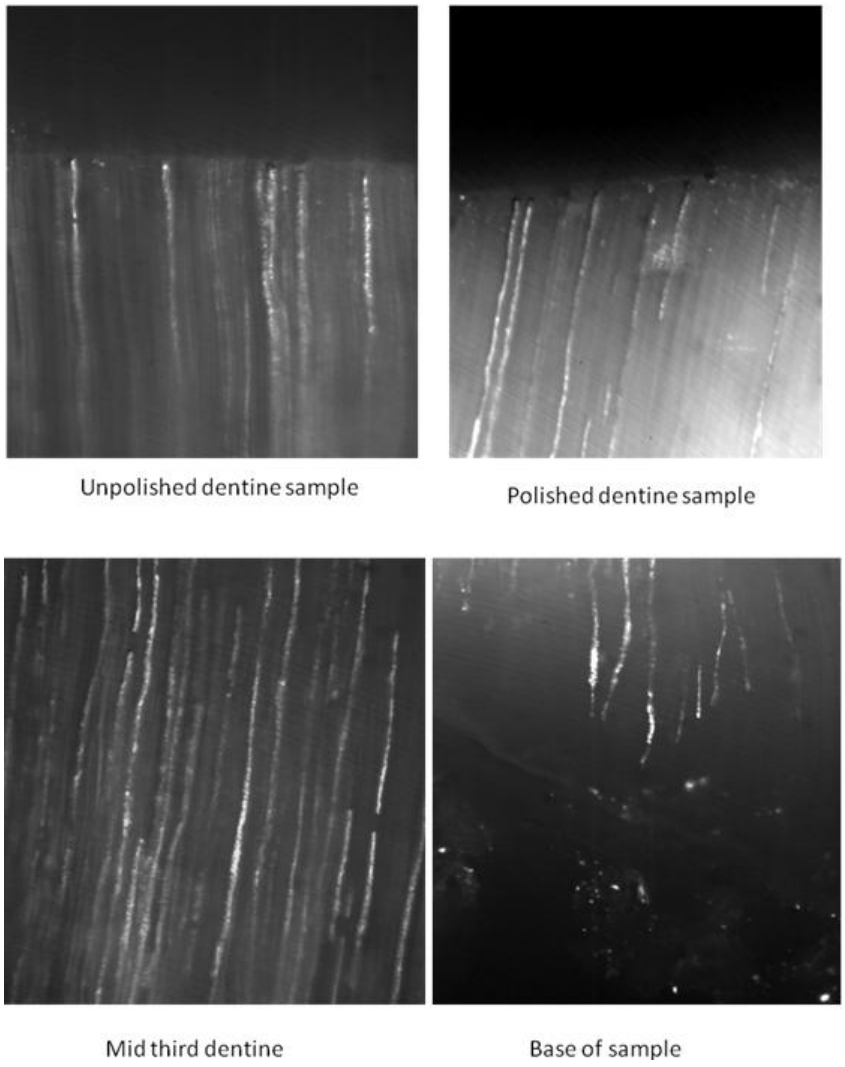


Figure 15 TSM using an oil based objective (x40/0.55 NA) of cross sections of the surface of unpolished (top left), surface of polished (top right), mid third of polished dentine (bottom left) and base of polished dentine samples (bottom right)

2.2.2.4 Pitfalls in dentine sample preparation observed using SEM and TSM

Chapters 4 and 5 of this thesis involve preparation of dentine samples with un-occluded dentine tubules. Figure 16 and Figure 17 show SEM and TSM images respectively of the surface of dentine samples, which were excluded from these studies due to poor sample orientation, over etching or insufficient water irrigation. It can be

observed from these figures that insufficient water irrigation, both during polishing using a semi-automated polishing system (Vector LC Power Head, Buehler, Lake Bluff, Illinois, USA) and in-between etching with 6% citric acid and NaOCl, might lead to a surface deposit because the surface of dentine and tubules appear covered. Increasing the etching time beyond 1 minute, using stronger acid challenges and increasing revolutions/minute on the shaker beyond 30 (Stuart Scientific, Mini Orbital Shaker, SO5) each resulted in a rippled appearance of the dentine surface. Poor dentine sample integrity may have been due to dentine sclerosis or alternatively due to incorrect dentine sample orientation during mounting and polishing, which resulted in a reduction in the number of visible un-occluded dentine tubules.

The length of 6% citric acid challenge required to remove the smear layer was investigated on the same samples using 30 rev/min gentle agitation (Stuart Scientific, Mini Orbital Shaker, SO5) and the images taken using TSM are shown in Figure 18. This shows that at baseline, the dentine surface is partially covered with a smear layer. Then following immersion in 6% citric acid for 30 seconds, some dentine tubules are visible. Following 60 seconds of etching, the dentine surface showed the presence of evenly distributed dentine tubules. However, longer acid challenges resulted in a change in the appearance of the etched dentine surface. TSM evaluation revealed an overall rougher appearance with smoothing of the inter-tubular regions. This created an undulating or rippled appearance at 80 seconds acid challenge. Then at 100 seconds of acid challenge some pitting was noticed on the surface. The pitted and rippling effect become more pronounced by 120 seconds. Similar effects were seen with increasing the agitation using the shaker beyond 30 rev/min with 1 minute of gentle agitation. Thus the acid challenge to expose un-occluded dentine tubules was limited to 1 minute using 6% citric acid and 30 rev/min agitations (Stuart Scientific, Mini Orbital Shaker, SO5).

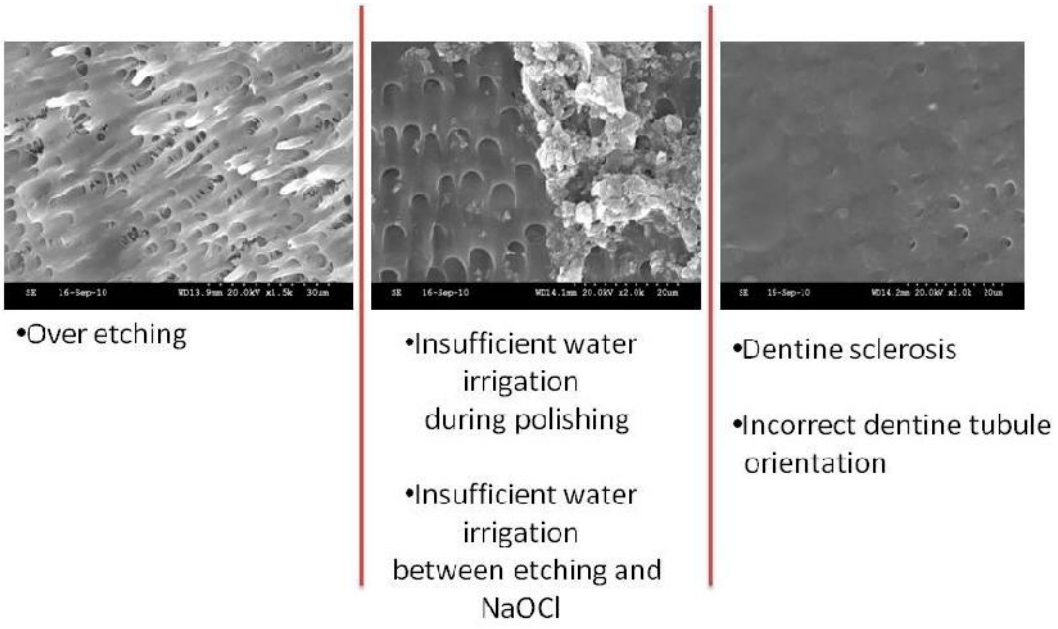


Figure 16 SEM of samples excluded due to over etching (left), insufficient water irrigation (middle) or poor dentine sample integrity (right) during processing

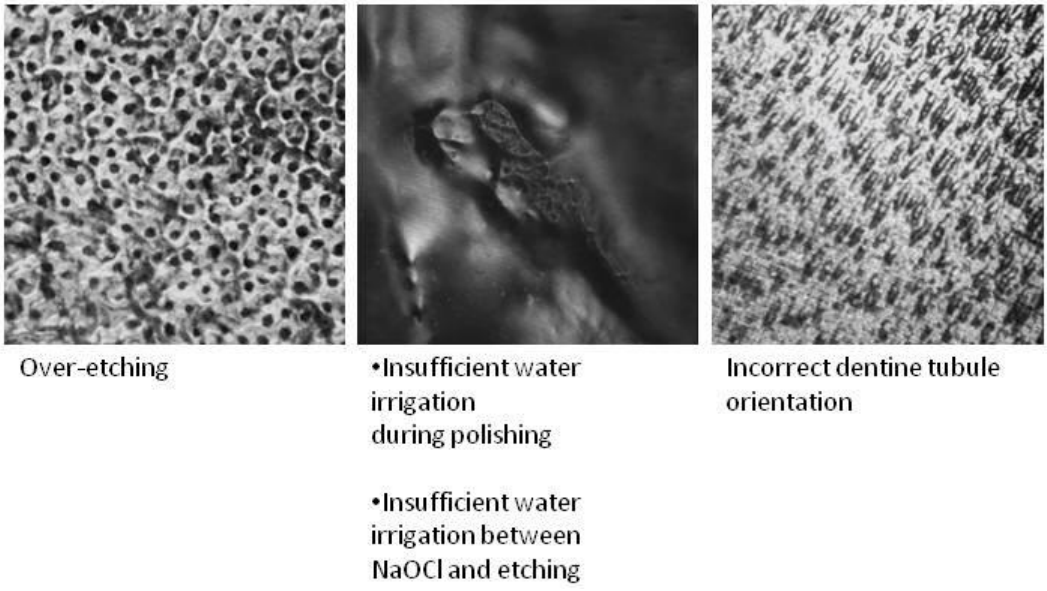


Figure 17 TSM (x40/0.55 NA dry lens) of dentine samples excluded due to over-etching (left), insufficient water irrigation (middle) or poor dentine sample integrity (right) during processing

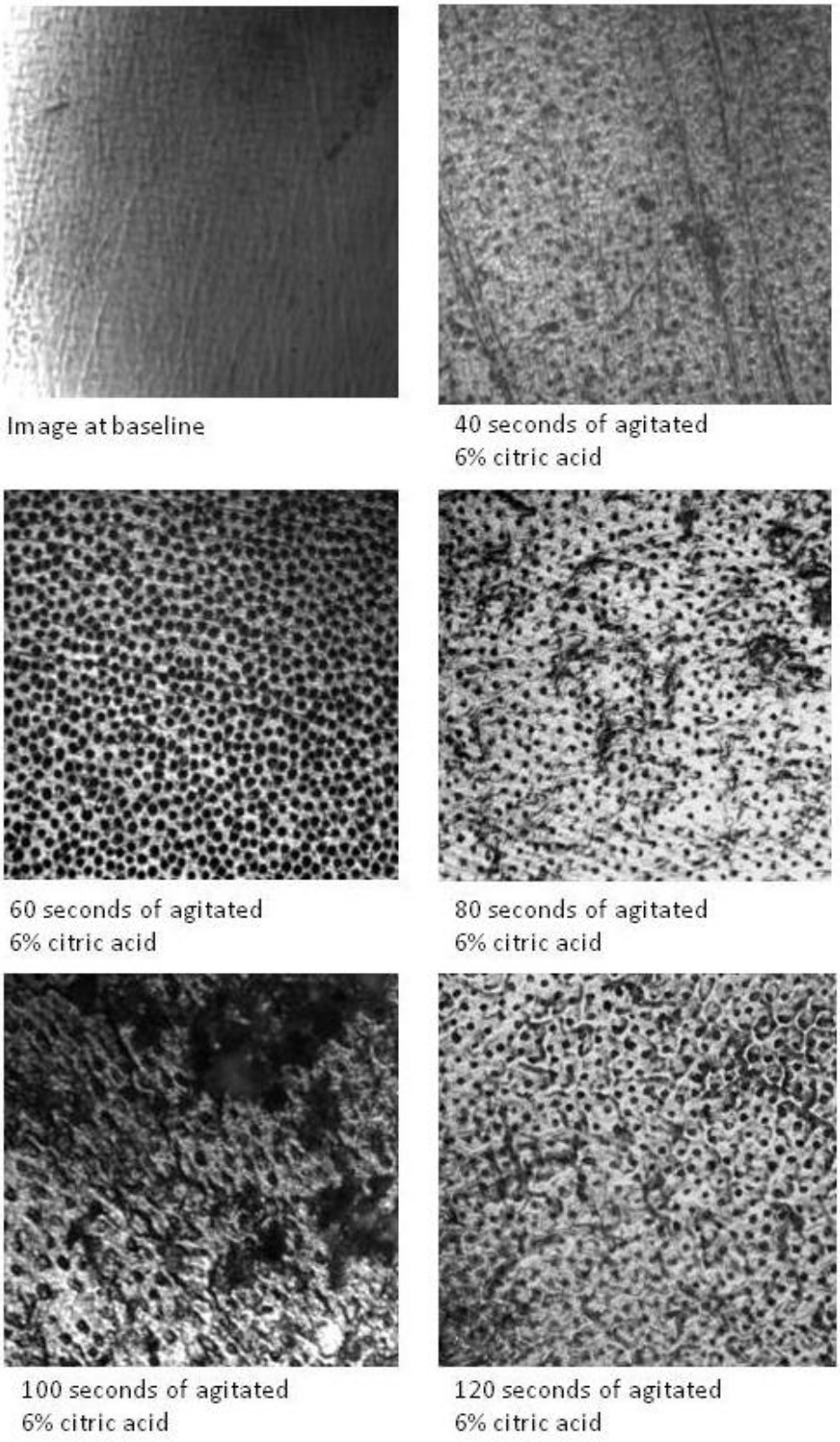


Figure 18 TSM (x40/0.55 NA) dry lens of the same dentine sample with 6% citric acid challenge under gentle agitation for various time lengths

Figure 19 shows the final appearance of the dentine surface with TSM and SEM, which were prepared ready for use. All dentine samples used in the study were prepared to an appearance similar to these. Samples used in the studies in Chapters 4 and 5 of this thesis were all screened using Tandem Scanning Confocal Microscopy (Noran Instruments, Middleton, WI, USA). The SEM processing stages rendered the sample unusable for subsequent use *in situ*.

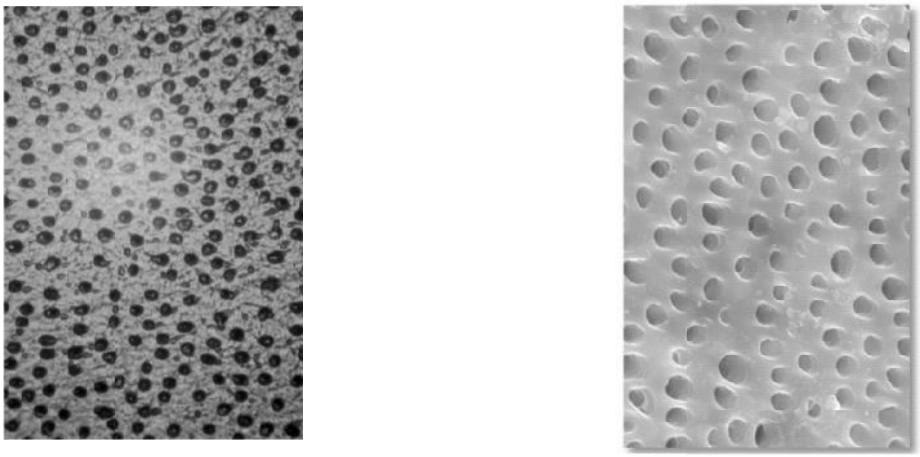


Figure 19 TSM x40/0.55 NA dry lens (left) and SEM x2000 (right) of the surface of dentine samples

2.2.2.5 Scanning electron microscopy protocol

The protocol described here was used in all studies involving SEM analysis of samples in this thesis. Samples were left to dry overnight and then placed in a specimen tray containing 24 wells prior to processing for SEM imaging. To ensure correct tracking of samples throughout the process, the sample number was written in indelible ink on the back of each well in the sample tray. Each sample was then mounted to a 0.5inch/12.5mm aluminium pin stub (Agar code G301), using Leit-C conducting carbon cement (Agar code G3300). The sample number was also written in indelible ink on the underside of the pin stub. Each set of 24 stubs was then placed into two 12-specimen storage boxes (Agar code G3103). The lids of the storage boxes were only lifted off as each specimen was placed in the box, and when a set of 12 was complete, all the

samples were lightly air-blown with a “Dust-Off Plus” can to remove any dust that might have settled during mounting.

Samples were then taken to the Centre for Ultra structural Imaging (CUI) at Guys Hospital for gold coating. During gold-coating the numbered underside of the stub was not affected. Once gold-coated, each sample was viewed using a S3500 Hitachi SEM. Six samples with pin stubs were attached in turn to a six-stub adapter and secured with grub screws. The adapter was placed in the viewing chamber of the SEM and the vacuum was then applied. Each specimen was imaged at the centre of the dentine sample, unless the centre was not representative of the general appearance of that dentine sample. The following settings were used for all samples:

Magnification: x2000

Working distance: 15mm

Accelerating voltage: 20 KV

The gold coating helped to reduce the effects of ‘charging’. To reduce this problem further during imaging, the voltage, beam current and or vacuum were reduced (Rice, 2012).

As each sample was imaged, its sample number was ticked on a sheet listing all the samples numbers, allowing any possible inconsistencies (missing or duplicated numbers) to be tracked back. Each image was saved in tiff format to the CUI image server, after which they were transferred onto CDs, which were provided as the master copy. The images were then saved to hard disk and to a backup server.

2.2.2.6 Tandem scanning microscopy protocol

The protocol described here was used in all studies involving TSM analysis of samples used in this thesis. The advantage was that samples could be imaged immediately using TSM and required no sample processing. Samples were taken to the Tracor Northern confocal microscope, with a mercury vapour illuminating source in the department of Biomaterials at Guys Hospital. They were then placed onto glass slides and imaged using a TSM x40/0.55 dry lens, unless otherwise stated. As an objective of the imaging is to detect un-occluded dentine tubules, a dry lens was used in order to detect surface particulate deposits that could otherwise occlude dentine tubules. Sub-surface imaging might not detect subtle changes to the dentine surface, which may be occluding the dentine tubules. Each image was saved onto the hard drive and then transferred to CD as the master copy. The images were then saved to hard disk and to a backup server.

2.2.2.7 Profilometry

To ensure imaging consistency in the studies in chapters 4 and 5 of this thesis, 10% of samples, selected randomly by computer, were assessed for surface topography measurement using a white light confocal profilometer (XYRIS 4000 WL, TaiCaan Technologies Ltd., Southampton, UK) to measure surface roughness.

Surface topographical measurement indicated the surface roughness on 10% of the samples was 0.5μm (SD 0.1μm). Profilometry showed that samples were polished to within 0.4-0.6μm flatness profiles. Within sample standard deviation in surface roughness has been reported in the literature as ± 0.06μm for enamel and ±0.09μm for dentine (Steiner-Oliveira *et al.*, 2010). Flat samples are beneficial for TSM imaging. In

addition, by reducing anatomical variation, they aid subsequent measurement of dentine tubules using visual or computational analysis routines. Other studies have investigated tooth wear and dentine tubule occlusion and have prepared the surface of dentine samples to produce a smooth surface (Banfield and Addy, 2004; Parkinson *et al.*, 2010; Parkinson and Willson, 2011a).

2.3 Training and calibration of examiners using the visual ordinal scale to measure dentine tubule occlusion

2.3.1 Introduction

Visual examination of the dentine surface using SEM or TSM was used to investigate the extent of dentine tubule occlusion. The visual ordinal scale is a standard method to analyse the amount of dentine tubule occlusion on dentine samples and was first described by Barlow *et al.*, 2007. The visual ordinal scale relies on training and calibration of examiners, to ensure it is reliable and accurate.

Using the scale three examiners graded the degree of dentine tubule occlusion observed on SEM images taken of the surface of dentine samples (Table 15). This relied on a brief visual assessment of the number, size and distribution of patent or un-occluded dentine tubules over the surface of the dentine on each image. A score of 0 indicated that the image is non-evaluable, for example due to wrong orientation of the dentine tubules. A score of 1 (occluded) indicated dentine tubules were not visible, score 2 (mostly occluded) indicated some dentine tubules were visible but most appeared occluded. A score 3 (equal) indicated a 50-50 spread of occluded and un-occluded dentine tubular space across the image. A score 4 (mostly un-occluded) indicated dentine tubules were visible but that the surface appeared partially occluded.

A score of 5 (un-occluded) indicated dentine tubules were visible, distributed throughout the image and spaced closely together. After all images were graded, the mean value of the examiners was calculated for each image.

This scale relies on training and calibration of the examiners. The training and calibration procedure will be described in this section.

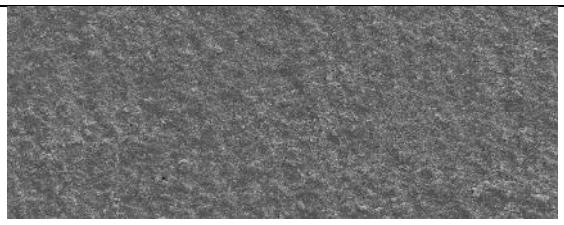
2.3.2 Samples

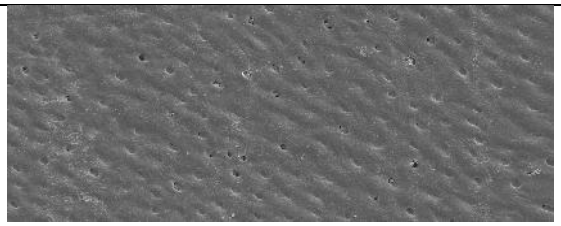
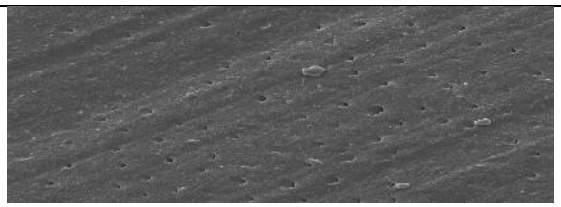
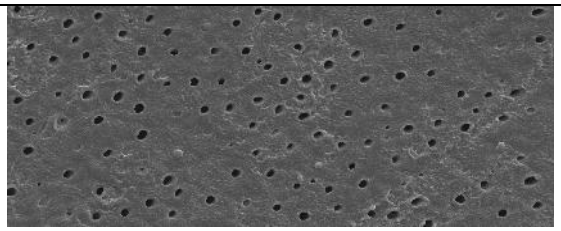
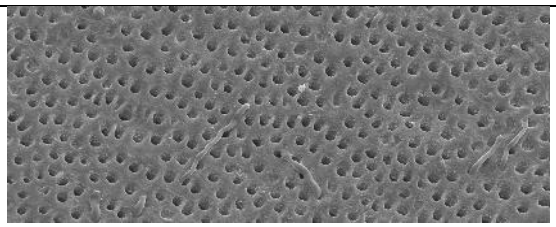
This procedure involved preparation of 60 dentine samples. These samples were then imaged using TSM and then SEM as described in sections 2.2.2.5 and 2.2.2.6.

2.3.3 Method

Four examiners were selected from the department of Restorative Dentistry at KCLDI and trained. The visual ordinal scale in Table 15 was explained in a power point presentation (Microsoft Office PowerPoint 2007).

Table 15 Visual ordinal scale for dentine tubule occlusion of SEM images

Grade	Description	SEM images of the surface of dentine samples (x2000)
1	Occluded	

Grade	Description	SEM images of the surface of dentine samples (x2000)
2	Mostly occluded	
3	Equal	
4	Mostly un-occluded	
5	Un-occluded	

2.3.3.1 Calibration of examiners using the visual ordinal scale for SEM images

A calibration training exercise was performed for dentine tubule occlusion classification scoring of SEM images using the visual ordinal scale, which were used in the studies in Chapters 4 and 5 of this thesis, prior to SEM image grading. The four examiners independently graded 30 SEM images. The scores from each examiner were cross tabulated against the experienced examiners scores. Then, in order to assess

agreement between each examiner and the experienced examiner, a Kappa coefficient (κ) was calculated to assess intra-examiner reliability and presented with 95% confidence intervals. Reliability was deemed excellent if κ >0.75, fair to good if 0.4≤ κ ≥0.75 or poor if κ <0.4.

2.3.3.2 Results of calibration training using visual ordinal scale for SEM images

The results for the four examiners for the study in Chapter 4 are shown in Table 16.

Table 16 Summary of intra-examiner calibration results for SEM visual ordinal scale Chapter 4

Examiner	Kappa Coefficient [95% CI]	Interpretation
1	0.90 [0.81, 0.99]	Excellent
2	0.92 [0.85, 1.00]	Excellent
3	0.82 [0.60, 1.00]	Excellent
4	0.60 [0.16, 1.00]	Fair to good

Three of the four examiners scored excellent (κ >0.75) on the Kappa scores and were subsequently selected to grade SEM images in Chapter 4 this thesis.

For the second study in Chapter 5 of this thesis, the same three examiners who scored 'Excellent' in Table 16 were trained a second time and asked to score the same images again. In addition, a fourth examiner was recruited who also had experience in SEM from the department of Restorative dentistry at Guys Hospital. The results for the four examiners for the study in Chapter 5 are shown in Table 17.

Table 17 Summary of intra-examiner calibration results for SEM visual ordinal scale Chapter 5

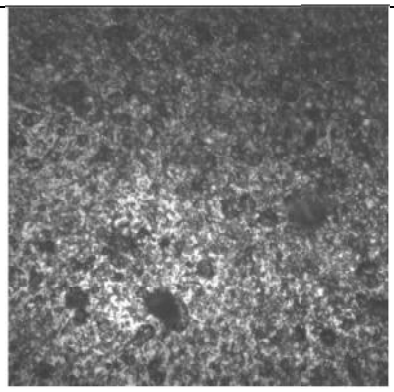
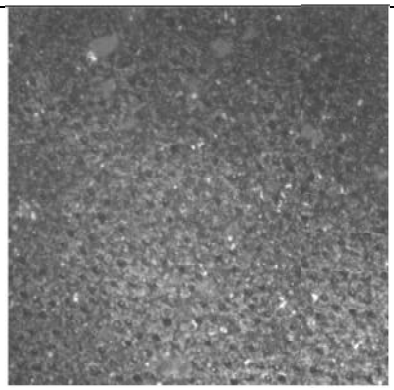
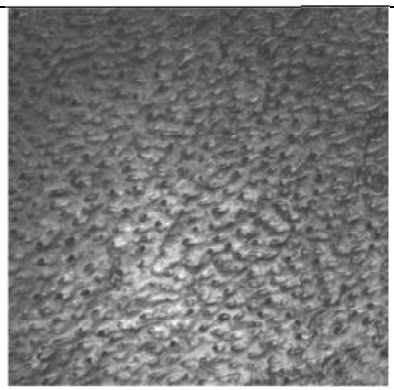
Examiner	Kappa Coefficient [95% CI]	Interpretation
1	0.90 [0.85, 1.00]	Excellent
2	0.93 [0.85, 1.00]	Excellent
3	0.92 [0.80, 1.00]	Excellent
4	0.60 [0.18, 0.99]	Fair to good

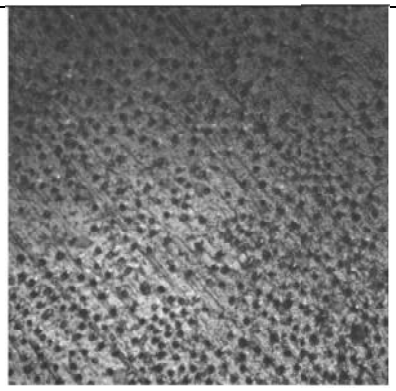
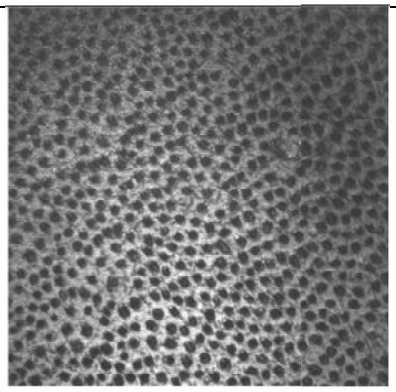
Three of the four examiners scored excellent (κ >0.75) on the Kappa scores and were subsequently selected to grade SEM images in Chapter 5 of this thesis.

2.3.3.3 Calibration of examiners using the visual ordinal scale for TSM images

A novel visual ordinal scale was designed for TSM image grading for use in Chapter 5 of this thesis and based on the SEM scale. Equivalent TSM images were taken of the samples used in Table 15, but for the purposes of grading TSM images (Noran Instruments, Middleton, WI, USA) in conjunction with an M-Plan 40x SLWD Bright field objective. These are shown in Table 18. Three examiners from the SEM training in section 2.3.3.1 graded 30 TSM images independently. An experienced examiner also graded the same images. The scores from each examiner were cross tabulated against the experienced examiners scores. Then, in order to assess agreement between each examiner and the experienced examiner, a Kappa coefficient (κ) was calculated to assess intra-examiner reliability and presented with 95% confidence intervals. Reliability was deemed excellent if κ >0.75, fair to good if 0.4≤ κ ≥0.75 or poor if κ <0.4.

Table 18 Visual ordinal scale for dentine tubule occlusion of TSM images

Grade	Description	TSM images of the surface of dentine samples (x40)
1	Occluded	
2	Mostly occluded	
3	Equal	

Grade	Description	TSM images of the surface of dentine samples (x40)
4	Mostly un-occluded	
5	Un-occluded	

2.3.3.4 Results of calibration training using visual ordinal scale for TSM images

The results for the three examiners are shown in Table 19.

Table 19 Summary of intra-examiner calibration results for TSM visual ordinal scale

Examiner	Kappa Coefficient [95% CI]	Interpretation
1	0.31 [0.18-0.49]	Poor
2	0.33 [0.17-0.47]	Poor
3	0.20 [0.12-0.34]	Poor

All three examiners scored poorly (κ <0.40). Therefore, I changed the ordinal scoring scale from six (0-5) to four (0-3) and re-trained three examiners on scoring the images. A score of 0 indicated that the image was non-evaluable. A score of 1 indicated the

image was occluded. A score of 2 indicated it was equal and a score of 3 indicated it was un-occluded. This shorter scoring system therefore removed the grades for mostly occluded or mostly un-occluded. The scores from each examiner were cross tabulated against the experienced examiners scores and a weighted Kappa coefficient (κ_w) calculated using the Fleiss-Cohen method of weighting (Fleiss and Cohen, 1973). Reliability was deemed excellent if $\kappa > 0.75$, fair to good if $0.4 \leq \kappa \geq 0.75$ or poor if $\kappa < 0.4$. The results for the three examiners are shown in Table 20.

Table 20 Summary of intra-examiner calibration results for TSM shortened visual ordinal scale

Examiner	Kappa Coefficient [95% CI]	Interpretation
1	0.68 [0.61-0.78]	Fair to good
2	0.46 [0.38-0.55]	Fair to good
3	0.61 [0.52-0.70]	Fair to good

In addition, agreement between examiners was also assessed using weighted Kappa and is shown in Table 21. Overall inter-examiner kappa was 0.243.

Table 21 Summary of inter-examiner calibration results for TSM shortened visual ordinal scale

Examiner	Kappa Coefficient [95% CI]	Interpretation
1 vs. 2	0.28 [0.10-0.40]	Poor
1 vs. 3	0.46 [0.38-0.55]	Fair to good
2 vs. 3	0.18 [0.09-0.27]	Poor

Agreement with the experienced examiner was not high but acceptable for examiner 1 and 3. However, examiner 1 and 3 had fair to good agreement with each other.

Examiner 2 did not agree particularly well with the experienced examiner or the other examiners.

2.3.4 Discussion

It was concluded that visual interpretation of the TSM images (taken at x40) was more difficult than the SEM images (taken at x2000). This is reflected in a poor intra-examiner agreement using the visual ordinal 'standard' to measure TSM images ($\leq$0.3) compared to SEM (>0.7) during calibration. Using a shortened visual ordinal scale with only 3 categories helped, but it was not possible to achieve excellent agreement.

2.4 Computational analysis to measure dentine tubule occlusion

2.4.1 Introduction

A visual count of each dentine tubules on every image would be particularly time consuming and probably subjective as described in section 1.23. In an earlier review, it was suggested that a digital-image-based analysis might be a more sensitive and accurate way of quantifying tubule occlusion (Grenby, 1996). The visual ordinal scale is based on a categorical scale 0-5 and is therefore limited in the amount of information it can provide in terms of tubule number density. It was therefore decided to write a computer software algorithm in order to count the number of un-occluded dentine tubules on SEM and TSM images. The aim of this section was to validate an innovative high resolution computerised method to measure dentine tubule occlusion using SEM and TSM.

2.4.2 Method

Ten dentine samples were prepared following the same protocols as described in section 2.2. Images were taken from the centre of each dentine sample using TSM (x40/NA 0.55 dry lens) and then SEM (x2000) as described in 2.2.2.6 and 2.2.2.5. Two computer software algorithms were designed in Image J (1.45s, Wayne Rasband, National Institutes of Health, USA) to count the number of un-occluded dentine tubules on each of the same 10 SEM and TSM images. These algorithms consisted of a series of macros, which were written to load each image, set up the measurements to be made, threshold the black areas on each image, make measurements of the threshold regions, save measurements and then repeat again for the next image. Software analysis counted all dentine tubules greater than a diameter of 0.83μm and excluded tubules overlapping the edges of images. This diameter was chosen based on the average diameter of dentine tubules reported in sensitive areas of recently extracted teeth (Absi *et al.*, 1987). A 0.83μm diameter is also a realistic diameter in order to be visually identified on a TSM or SEM image. In addition, previous work has discussed that dentine tubule recorded as a diameter is independent from tubule orientation (Arends *et al.*, 1995; Schilke *et al.*, 2000), which is particularly relevant considering that variation in the size of untreated tubules and their density throughout the tooth is highly varied (Mjor and Nordahl, 1996). Figure 20 shows a TSM image of a dentine sample with un-occluded dentine tubules and shows a dentine tubule of 0.83μm circled. Figure 21 shows a SEM image of a different dentine sample with un-occluded dentine tubules and shows a dentine tubule of 0.83μm circled.

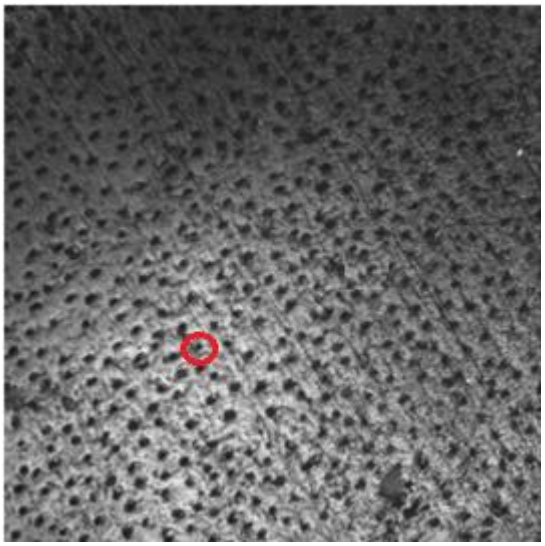


Figure 20 TSM (x40/0.55 NA dry lens) showing a dentine tubule of 0.83 µm diameter (circled)

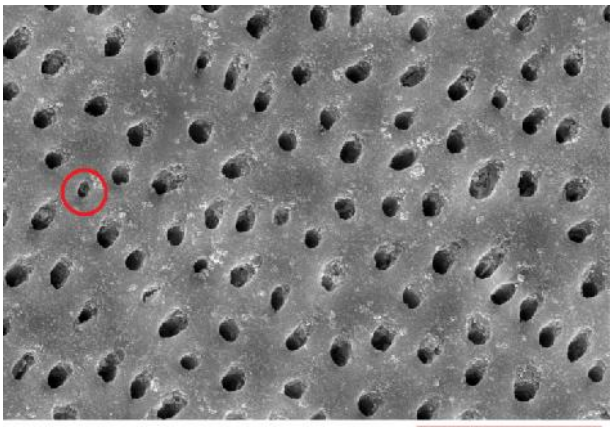


Figure 21 SEM (x2000) showing a dentine tubule of 0.83 µm diameter (circled)

The macro algorithm for the TSM is outlined below;

1. Locate image files in a specific folder on the hard drive,
2. Polynomial regression in which the relationship between the independent variable *x* and the dependent variable *y* is modelled as an *n*th order polynomial,
3. Gaussian blur to reduce image noise and reduce detail,
4. Use of inter-pixel relationship and intensity to find the edges of the un-occluded

dentine tubules,

5. Partitioning a digital image into multiple segments to locate objects and boundaries in the images,
6. Removal of the background (intertubular dentine),
7. Conversion of the image into binary format using image thresholding in which individual pixels in an image are marked as "object" pixels if their value is greater than some threshold value (assuming an object to be brighter than the background) and as "background" pixels otherwise,
8. To highlight un-occluded dentine tubules in preparation for analysis,
9. Select the whole area of the image,
10. To count the number of objects representing un-occluded dentine tubules, greater than 0.83μm diameter of any circularity,
11. To close all windows and repeat the same procedure on the next image.

The macro algorithm for the SEM is outlined below. The macro is very similar to that used for the TSM, but there are a few differences. Sentence 4 involves removing outliers in the image (or pixels which are of a colour intensity numerically distant from the rest of the data) and “despeckle” (in order to remove noise from the image without blurring them). These were primarily designed in order to overcome the charging effects, which were realized on some of the SEM images, as described in section 1.17.1. In addition, it was not necessary to ‘remove background’ in the SEM image.

1. Locate image files in a specific folder on the hard drive,
2. Polynomial regression in which the relationship between the independent variable *x* and the dependent variable *y* is modelled as an *n*th order polynomial,
3. Gaussian blur to reduce image noise and reduce detail,
4. Remove outliers and despeckle,

5. Use of inter-pixel relationship and intensity to find the edges of the un-occluded dentine tubules,
6. Partitioning a digital image into multiple segments to locate objects and boundaries in the images,
7. Conversion of the image into binary format using image thresholding in which individual pixels in an image are marked as "object" pixels if their value is greater than some threshold value (assuming an object to be brighter than the background) and as "background" pixels otherwise,
8. To highlight un-occluded dentine tubules in preparation for analysis,
9. Select the whole area of the image,
10. To count the number of objects representing un-occluded dentine tubules, greater than 0.83μm diameter of any circularity,
11. To close all windows and repeat the same procedure on the next image.

A further thirty dentine samples (section 2.2) were then prepared in order to calibrate the software. Images were taken from the centre of each dentine sample using TSM (x40/NA 0.55 dry lens) and SEM (x2000) as described in 2.2.2.5 and 2.2.2.6 above. The total number of dentine tubules and the maximum diameter of dentine tubules were then recorded visually on the thirty SEM and TSM images. Figure 22 shows a TSM image (x40) and a traced drawing of the same TSM image showing un-occluded dentine tubules. The un-occluded dentine tubules in each image were traced as shown and then the total number of un-occluded tubules was recorded for each image. The procedure was repeated for the SEM images. In addition, the diameter was measured for the dentine tubules using a graduated rule and the value averaged for each image.

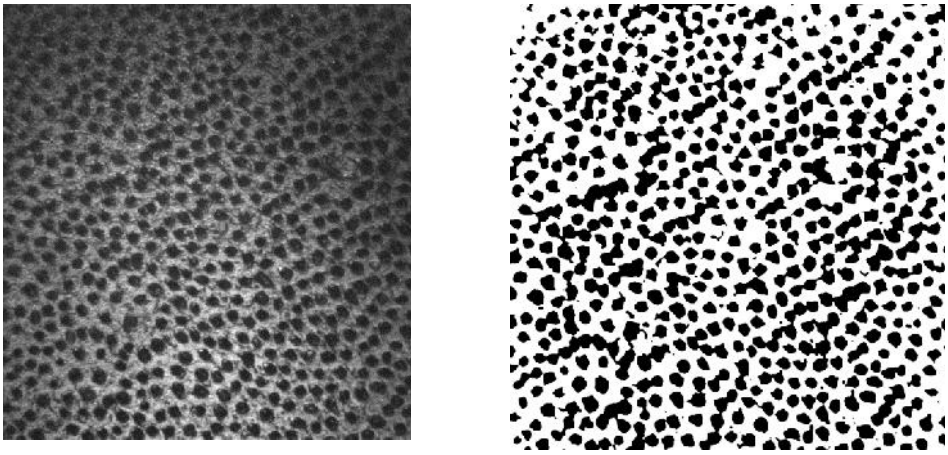


Figure 22 TSM image (x40/ 0.55 NA dry lens) (left) and traced drawing of un-occluded dentine tubules (right)

This data on the number of un-occluded dentine tubules and diameter of tubules was then cross tabulated with the number and maximum diameter of dentine tubules recorded using the software.

2.4.3 Results

Reproducibility between the visual count of un-occluded tubules and the number of un-occluded dentine tubules counted using the software assessment for TSM and SEM images respectively was then assessed using intra-class correlation coefficients and was $\geq$0.9. The same exercise, but this time using an average diameter of un-occluded dentine tubules estimated by visual counting and then by the software was $\geq$0.8.

2.4.4 Discussion

Intra-class agreement of the computational analysis to the numbers of un-occluded dentine tubules counted visually was $\geq$0.8 for SEM and TSM images. Previous *in vitro* work demonstrated similar correlations using SEM (Ciocca *et al.*, 2007). It has been

possible to design a software macro to accurately count the number of un-occluded dentine tubules and a computerised method to measure un-occluded dentine tubules on TSM images is novel. An automated computational analysis routine also allows identical interpretation of images and this software could be applied to studies investigating agents designed to occlude dentine tubules. However, it has also not yet been correlated to an existing method of measuring dentine tubule occlusion in the literature, for example the visual ordinal scale.

2.5 Section three: Clinical study design for investigation of dentine tubule occlusion

2.5.1 *In situ* studies

In chapters 4 and 5 of this thesis, *in situ* randomized control studies were designed to investigate the effect of desensitising dentifrices on dentine tubule occlusion.

2.5.2 Appliance design

Several *in situ* studies have been conducted which have investigated dentine tubule occlusion. These have involved use of intra-oral appliances, most commonly held in the palate or on the buccal aspect of the lower posterior dentition. Previous work has investigated the effect of the soft tissues on dental wear and DH. One *in situ* study showed that the tongue could exert an abrasive effect on dental tissues softened by erosion (Gregg *et al.*, 2004). Therefore, a study investigating the effects of acid challenge and abrasion on dentine tubule occlusion would require that the samples are worn in areas away from the action of the tongue. Consequently, it was decided to design appliances to hold dentine samples in buccal oral appliances.

A crossover design was chosen in order to allow each subject to act as their control and therefore reduce variability. Therefore, a buccal appliance was designed for each posterior buccal segment and colour coded according to the side of mouth. Figure 23 show intra-oral mandibular buccal appliances designed for an *in situ* study to investigate dentine tubule occlusion, which were designed based on previously published studies (Banfield and Addy, 2004; Claydon *et al.*, 2009).

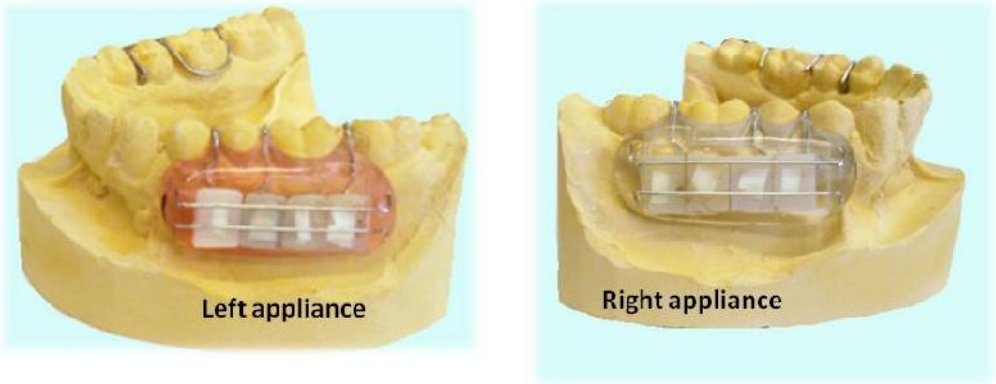


Figure 23 Intra-oral mandibular buccal appliances

2.5.3 Abrasion protocol

The abrasion protocol was designed using a desensitising dentifrice and applying this at various time points to the dentine samples. The dentine samples in each appliance had a different product applied. It was important to ensure that the same person administered the dose of product for a particular subject throughout the study duration to limit variations in brushing technique.

In order to assess a number of products on the dentine, a washout period was required in between each product application. This was to ensure there were no carry over effects of one product used on dentine samples.

2.5.4 Acid challenge protocol

The mean titratable acidity and pH following five repeat measurements of 20ml of a variety of popular erosive beverages was assessed with 0.1Mol NaOH using a calibrated pH bench top meter and electrode (Oakton pH 510 meter and WD-35801-00 pH electrode, Eutech Instruments, Nijkerk, Netherlands). The results are shown in Table 22 below.

Table 22 Titratable acidity and pH of erosive beverages

Erosive beverage	**Mean titratable acidity (ml)**	**Mean pH**
Tesco Pure grapefruit juice (Smooth)	44.70	3.49
Sainsbury's apple juice (Smooth)	37.80	3.70
Sainsbury's orange juice (Smooth)	22.74	3.58
Coca-Cola regular	21.10	2.40
Red Wine (Tesco Chile Merlot)	21.70	3.50
Beer (Fosters Lager)	10.10	4.40

Tesco Pure grapefruit juice had the highest titratable acidity. The results confirmed previous studies (Addy *et al.*, 1987a; Grenby *et al.*, 1989). Grapefruit juice contains grapefruit from concentrate (with a citric acid concentration of 2.5% (Penniston *et al.*, 2008)). Unlike citric acid, the use of grapefruit juice represents a typical acid challenge that may also be experienced within society.

It was therefore decided to use a Tesco Pure grapefruit juice (Smooth) under gentle agitation for the acid challenge protocol in the clinical studies. It has been used previously in studies *in vitro* to investigate dentine tubule occlusion (Parkinson *et al.*, 2010), but without agitation and not *in situ*.

2.6 Conclusions

Numerous clinical and laboratory studies have been conducted to measure tooth wear and DH, as discussed previously in sections 1.15 and 1.16. Some of these studies collect data at the tooth surface level, whereas other studies collect data at the subject level. The BEWE and Schiff indices are both based on an ordinal scale between 0 and 3. The BEWE and Schiff tooth wear index, each recorded as a sextant cumulative score per subject, has been shown to provide a representation of the tooth wear and DH process respectively, recorded as either a percentage of tooth surfaces per patient or as a highest score per patient. This means that the sextant score for Schiff and BEWE are useful screening tools for assessing DH and tooth wear and which avoid the need for recording DH and tooth wear on every tooth surface respectively. They will also be adequate tools on which to compare risk factors, tooth wear and DH per subject, in Chapter 3 of this thesis.

Investigations of the mechanism of action of DH through dentine tubule occlusion require investigation of the surface of dentine. This requires a standardised sample preparation and processing procedure. A standardised procedure has been developed, which produces dentine samples with a 0.5μm (SD 0.1μm) surface roughness for subsequent investigation and imaging. Samples were prepared using a standardised methodology to reduce anatomical variation. Profilometry showed that samples were polished to within 0.4-0.6μm flatness profiles. Within sample standard deviation has been reported as ±0.09μm for dentine (Steiner-Oliveira *et al.*, 2010). Flat samples are beneficial for imaging and subsequent quantification of dentine tubules per image. Imaging acquisition was also standardised using high quality images and SEM and TSM images were taken consistently from the centre of each sample.

In order to then measure dentine tubule occlusion, the visual ordinal scale ('standard') has been calibrated to grade SEM images taken of the surface of dentine samples. The intra-examiner agreement was $\geq$0.7 using Cohen's kappa coefficient. Unfortunately, when the visual ordinal scale was applied to grade TSM images, the intra-examiner agreement was only 0.2-0.3 using Cohen's kappa coefficient. Therefore, the visual ordinal scale will be used to grade SEM images, but not TSM images.

An innovative high-resolution computerised method has also been developed to measure dentine tubule occlusion on SEM and TSM images. Intra-class agreement of the computational analysis to the numbers of un-occluded dentine tubules counted visually was $\geq$0.9 for SEM and TSM images. This shows that the computerised method is an accurate method on which to measure dentine tubule occlusion. It requires comparison to established methods used to measure dentine tubule occlusion, for example the visual ordinal scale ('standard'). Furthermore, use of a computerised technique to measure tubule occlusion on TSM images is novel and has not been applied to dentine discs used *in situ* to investigate agents designed to occlude dentine tubules and the aetiology of DH.

A protocol has been devised for an *in situ* study to investigate dentine tubule occlusion of dentifrices designed to treat dentine hypersensitivity. *In situ* models are commonly used in many oral therapeutic areas to help qualify *in vitro* performance and provide insight into clinical efficacy. They require dentine discs mounted in the oral cavity to subject them to the influence of the oral environment, but allow them to be studied using a surrogate approach (Hooper *et al.*, 2005; West *et al.*, 1997). Clinical trials are often conducted to measure DH and the effectiveness of punitive treatments, but they provide little information on the mechanism of action (Markowitz and Pashley, 2008).

The results of clinical trials are often confounded by many factors, for example variations in subject-based reproducibility of the degree of pain associated with DH, a subjects pain tolerance, despite standardisation of stimuli used to elicit DH (West *et al.*, 1997). Critically, *in vivo* studies, provide only limited information on the mechanism of action and occlusion potential of treatments used for DH. Studies *in vivo* to measure the number of un-occluded dentine tubules and thus the occlusion potential of treatments are challenging. For example, *in vivo* imaging techniques do not have the required stability to acquire images of dentine tubules at the required resolution (Watson *et al.*, 1992). In contrast, *in situ* studies have been widely employed in the study of conditions that affect dental hard tissue surfaces, for example dental erosion (Zero, 1996), caries (Zero and Lussi, 2005) and DH (Addy, 2002) and importantly permit *ex vivo* analysis of the tissue under investigation.

The *in situ* protocol has been designed to have a strong acid component using an agitated acid challenge to reflect the importance of erosion in the aetiology of DH. Grapefruit juice was also shown to have a higher titratable acidity than many other popular erosive beverages and in addition a shaker/stirrer has been shown to create pronounced effects on the surface of dentine. The use of an agitated acid challenge has been shown to enhance erosion and may be more representative than still challenges because the solution in contact with the dental tissue is readily removed (Shellis *et al.*, 2005). An agitated acid challenge does not take into account the buffering of intakes as would be expected in the oral cavity and whilst extreme it might be considered representative of the acidic challenges that a person may expect to receive following frequent and large consumptions of acidic beverages. Therefore, it could be regarded as a strong acid challenge, which might typically be consumed in the UK.

Chapter 3 Prevalence of DH and tooth wear and associated risk factors

3.1 Introduction

DH, apart from having an impact on quality of life, might be a fundamental predictive risk factor for early detection of tooth wear and preventive interventions for these conditions (West, 2006) and patients often present to their dentist with signs of DH. It is generally agreed that the aetiology of tooth wear and DH is multi-factorial and identification of aetiological factors will determine the prognosis of the tooth wear and DH and suggest the treatment plan (Dababneh *et al.*, 1999). However to the author's knowledge, there are few studies linking the presence or absence of DH in association with tooth wear and various aetiologies. Better knowledge of DH would help establish preventive measures that seek to reduce the incidence of this condition and to diminish its impact, given that the condition has functional, aesthetic and painful consequences that impact on the quality of life of adult sufferers (Bekes *et al.*, 2009; Boiko *et al.*, 2010).

It is possible to understand the aetiology of tooth wear lesions and DH by identifying and analysing certain potential causal factors. These determining factors may be gleaned from:

- Questionnaires on hygiene habits, dietary habits and any pathology or medication that could modify the pH of the oral environment or affect the buffering capacity of saliva.
- Clinical records, which provide information from ordinal indices on tooth wear, DH and periodontal health.

3.2 Aim

The proposed study is a cross sectional and multi-centre study investigating the prevalence and aetiology of tooth wear and DH on all tooth surfaces (buccal, occlusal/incisal and lingual/palatal) and associated risk factors.

The steps used to validate the tooth wear and DH indices were described in Chapter 2 (section 2.1). In this Chapter, the BEWE and the Schiff sextant cumulative scores were used to record the severity of tooth wear and DH.

A number of objectives of Chapter 3 include;

1. Validation
    - Is there a correlation between the number of tooth surfaces recorded to have DH using the DH index per subject and the number of tooth surfaces recorded to have DH using the Schiff index per subject?
    - Is there a correlation between the clinical data outcomes (Schiff and BEWE sextant cumulative scores) for the 10% of randomly selected subjects who were examined twice?
2. Subject reported DH
    - Is there a difference between subjects reported DH in the questionnaire and with that recorded at the clinical examination?
    - Do the outcomes for subject reported anxiety, depression and life events correlate with reported DH?
3. Is there a relationship between the BEWE sextant cumulative score and SCHIFF sextant cumulative score recorded at the clinical appointment and aetiological

factors including brushing, frequency of consumption of acidic foods and drinks and various life events?

4. Is there a relationship between the Schiff sextant cumulative score and the BEWE sextant cumulative score?
5. The relationship between the side of mouth brushed (right/left) and the 'left' and 'right' percentage 'recession', 'left' and 'right' percentage BEWE scores and left and right percentage Schiff scores respectively.
6. Description of tooth wear, DH and aetiology data for all data and separately for BEWE groups 1, 2 and 3.

This study was run as part of a larger cross sectional and multi-centre epidemiology European study which aimed to identify risk factors for NCCLs as diagnosed in dental practice. This encompasses twelve countries in Europe including two centres in the UK (one at KCLDI and another at Bristol). This study involves a subject and clinical questionnaire, with slightly more questions on the subject questionnaire compared to the European study. In addition, the European study is collecting data from buccal and lingual/palatal tooth surfaces only. It was decided in this study to also collect DH (and tooth wear) data from occlusal/incisal tooth surfaces. This is because tooth wear also affects occlusal/incisal surfaces and there is a contribution from erosion as well as attrition and abrasion on these surfaces (Bartlett, 2005b). To the authors knowledge, few studies have been conducted to measure DH on occlusal in comparison with cervical tooth surfaces.

Some of the results of this study will be used as part of the European study.

3.3 Null hypotheses

The null hypothesis is that there is no association between tooth wear, DH and risk factors.

3.4 Method

The sample size was set by the European lead for the purposes of investigating the effect of aetiological factors on tooth wear and DH. With two controls for each tooth wear lesion, an expected odds ratio of 2, a risk of 5% and a power of 80%, the required number of participants would be 332. The estimated error margin of 5% (incomplete questionnaires) brought the required number of participants to 350. The selection of sites aimed to include urban, suburban and rural populations. Eight sites were invited to take part in the study, including three NHS hospital sites and five private/NHS dental practices in SE England. Four of these sites were inner city (metropolitan sites), two were suburban (town sites) and two were in small town/rural sites. An equal sample number of subjects were recruited at each site. The ethical approval (11/H0801/3) was granted by South West London Research Ethics Committee. Following this, agreement was sought from the relevant local health authorities and research and development sites.

A convenience sample was then taken by recruiting patients sequentially at each site who were willing to participate in accordance with the eligibility criteria. Eligibility criteria are included in section 2.1.2.5. For every person included in the study, the procedure was first explained to the participant. They were also provided with a patient information sheet following which the consent form was signed. The patient information

sheet and consent form are available on request. Subjects who consented to the study and fulfilled requirements were asked to complete the questionnaire. This questionnaire was developed in consultation with an expert scientific committee comprising senior clinical academics in restorative dentistry across Europe. It was designed to be self-administered although it was completed as part of an interview questionnaire to assist the subjects in explanation of terminology and its completion (see section 7.1). The questionnaire included:

- Data on the participant's oral hygiene practices,
- The participant's Oral Health Related Quality of Life: this scale is a measurement tool comprising 7 questions (Boiko *et al.*, 2010); responses are made on a 4 point scale,
- Data on the participant's perception of DH: intensity, duration, origin,
- Data on the evaluation of risk factors associated with tooth wear lesions (tobacco, medication, diet),
- Data on the evaluation of risk factors associated with tooth wear lesions: seeking aid in the dental care system; health associated preventive behaviours (weight, size).

The questions on oral health related quality of life are included in the questionnaire. The questionnaire was piloted on ten subjects at KCLDI to ensure that the questions were easily understandable. In addition, when the study commenced, the questionnaire was undertaken in the style of an interview questionnaire to further enable subjects to answer each question. Training on how to provide interview questionnaires had been undertaken.

Then, BEWE scores were recorded on all tooth surfaces excluding 3rd molars and Schiff scores were recorded on all teeth excluding 2nd and 3rd molars (section 2.1). In

addition to BEWE and Schiff, the clinical questionnaire collected clinical data about periodontal conditions:

- Gingival recession on the buccal and palatally/lingual surfaces (mm),
- Depth of periodontal pockets (mm),
- Presence or absence of gingival bleeding.

For gingival recession, measurements were made using a 1mm graduated periodontal probe from the amelocemental junction to the free gingival margin. For periodontal pocket depth, measurements were taken from the free gingival margin to the base of the periodontal pocket. The clinical questionnaire is shown in section 7.2 and was designed using spreadsheet software (Microsoft® Office Excel® 2007, Microsoft® Corporation, USA). The clinical form was carried on a laptop and password protected to prevent unauthorised access. A master template was created and used to load a fresh spreadsheet for each subject recruited to the study. Data were then entered directly into the form. The spreadsheet was programmed to ensure only set values could be entered into each cell in order to avoid data entry errors. Thus for Schiff and BEWE, entry values were set as 0, 1, 2 or 3. For DH index, values were set as 0 or 1. For bleeding, values were 0 or 1. For loss of attachment and periodontal probing depth, a data entry value between 0-9 was programmed. In addition, for every subject, additional data was collected including;

- Date of examination,
- Subject ID,
- Site ID,
- Year of birth,
- Gender,
- Location (metropolitan/town/rural),

- Education (Self employed, managers, other white collars, manual workers, house person, unemployed, student).

The dentist at each site was not requested to modify their usual management practices for patients. The study did not alter the dentist–patient relationship. The dentist remained free to decide their treatment options and follow-up procedures and no out-of-the-ordinary treatment or examination was linked to this study.

Clinical examinations were repeated on a 10% of the sample to check for intra-examiner variability (as described in section 2.1.2.9). For convenience to the site and subject, this occurred at the same appointment.

Using the spreadsheet software, a number of formulas were input to calculate clinical outcomes for each subject;

- BEWE and Schiff scores per subject presented as sextant cumulative score, percentage score and highest score per subject (as described in section 2.1),
- Number of tooth surfaces with a positive score for the DH index (section 2.1.2.8),
- Number of tooth surfaces with a positive score for Schiff index (section 2.1.2.8),
- Percentage of tooth surfaces with recession per subject,
- Percentage of sites bleeding following periodontal probing per subject.

The qualitative variables and clinical outcomes were entered into a statistical package (IBM® SPSS® Version 20). The data were then described. The relationship between the side of mouth brushed (right/left) and recession, BEWE and Schiff respectively was also assessed. In this assessment, rather than use BEWE and Schiff sextant

cumulative scores, percentages for BEWE ≥1, ≥2 and Schiff ≥1, ≥2 on the left and right hand side of the mouth were used as variables for tooth wear and DH respectively. Differences between right and left hand side of the mouth were then compared.

The analysis was undertaken in two stages. First, a univariate analysis measured separately the strength of association between the disease (cumulative sextant scores for tooth wear and DH) and each of the exposure factors taken from the clinical and subject based questionnaires respectively, without taking account of other potential confounding factors. Factors with strong statistically significant ($p<0.05$) associations with tooth wear or DH (dependant variables) in the univariate analyses were then used in a multivariate analysis, otherwise known as a multiple linear regression model, as predictor variables. The predictors were inserted as independent variables. This model allows analysis of all the risk factors to be taken into account by simultaneously adjusting for their effects. Multiple logistic regression analysis is a standard analytical method for case control studies and allows adjusted coefficients (and their associated confidence intervals) to be estimated for each exposure factor.

The term tooth sensitivity is used in the subject questionnaires. However, for consistency, the term DH will be used throughout in the results and discussion.

3.5 Results

The clinical prevalence of tooth wear and DH recorded from this study using sextant cumulative scores, percentage scores on all tooth surfaces per subject and as highest scores per subject are summarised in section 2.1.3.

3.5.1 Demographics

A sample of 350 subjects was recruited from primary (62.6%, n=219) and secondary (37.4%, n=131) care sites in the south east of England between June 2011 and February 2012. A convenience sample of 43-44 consecutive subjects were obtained at each site. Subjects were aged between 19 and 34 years old (mean 26.76, SD 3.55, SE 0.19) as shown in Figure 24.

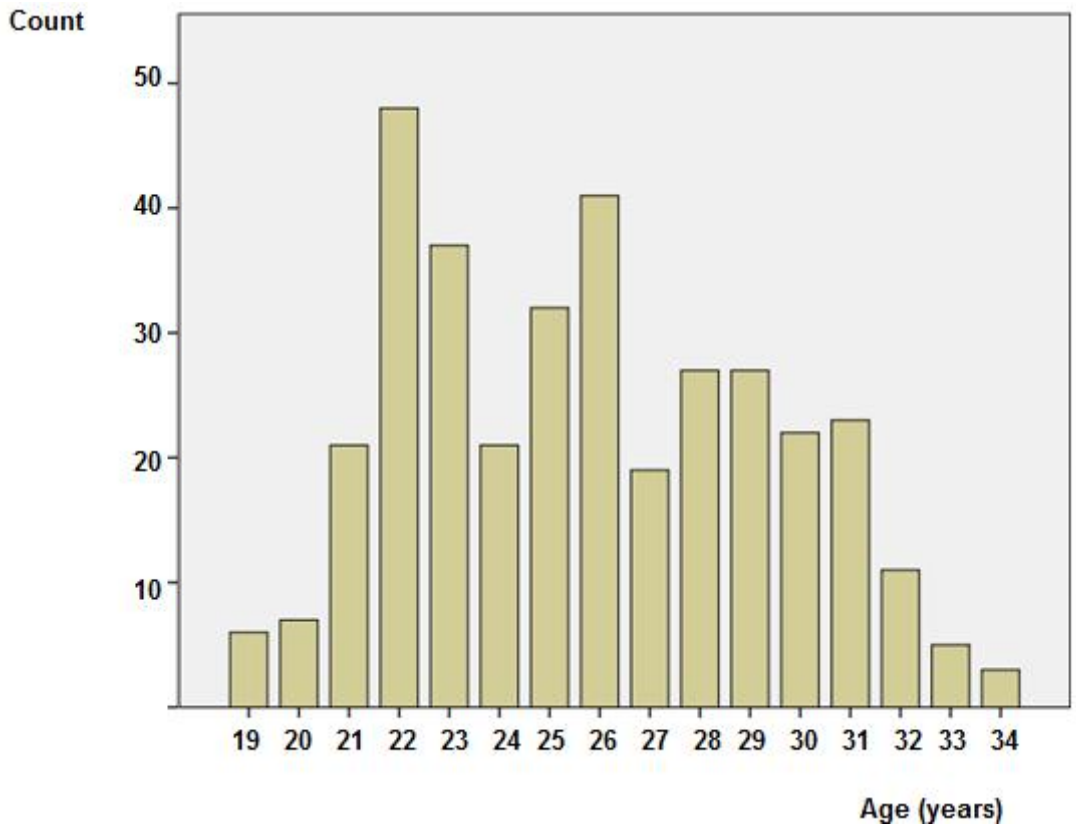


Figure 24 Age of subjects (n=350)

A total of 56.3% (n=197) were female and 46.4% (n=153) were male. Subjects reported living in metropolitan (35.7%, n=125), rural (10.9%, n=38) or small/mid-size town (53.4%, n=187) regions. Their level of education (in years of age) was 20+ (35.7%, n=125), 16-19 (34.3%, n=120), still studying (27.7%, n=97) or 15 (2.3%, n=8). Subjects who were employed (58.3%, n=204) included managers (6.3%, n=22), manual workers

(6.9%, n=24), other white-collar workers (27.1%, n=95) and self-employed (18%, n=63). The remainder included 25.2% (n=88) students, 8.9% (n=31) house persons and 7.7% (n=27) unemployed.

A total of 24, 093 tooth surfaces were examined and included 8, 053 buccal, 8, 014 occlusal and 8, 026 lingual tooth surfaces. Restored, carious and missing teeth accounted for 5, 307 tooth surfaces.

3.5.2 Validation

Reproducibility of all clinical outcomes for tooth wear and DH per subject on 10% of the randomly selected sample was $\geq$0.96. Reproducibility of scoring DH on every tooth surface using DH and then Schiff at the same appointment was $\geq$0.98. In section 2.1, the BEWE sextant cumulative score and Schiff sextant cumulative score were validated as clinical outcomes for the tooth wear and DH respectively in this study.

3.5.3 Tooth wear and DH recorded per subject

In total, tooth wear (or a BEWE score of 1 and above) occurred to some extent in 91% (n=318) subjects. Of these subjects, 81% (n=283) had wear on the occlusal/incisal surfaces, 73% (n=256) on buccal surfaces and 25% (n=88) on lingual/palatal tooth surfaces.

DH was recorded on at least one tooth surface at their clinical appointment in 43.4% of subject's (n=152). DH occurred in 43.1% (n=151) subject's buccal surfaces, 28% (n=98) subject's occlusal/incisal surfaces and 26% (n=91) subject's lingual/palatal tooth surfaces.

Subjects reported in the questionnaire having DH “often” in the previous 12 months (24.6%, n=86) or it was reported “often” or “occasionally” in the previous 12 months (59.7%, n=209). Less than half reported having DH at their clinical appointment (44.3%, n=155) and is similar to the proportion of subjects shown to have DH at their clinical appointment (43.4%, n=152).

The wear and DH recorded using the BEWE and Schiff sextant cumulative scores are shown in Figure 25 and Figure 26 respectively. By far the greatest DH was recorded using the Schiff sextant cumulative score in the 19 year old and 33 year old age groups. The data is more evenly spread over the ages for the BEWE sextant cumulative scores.

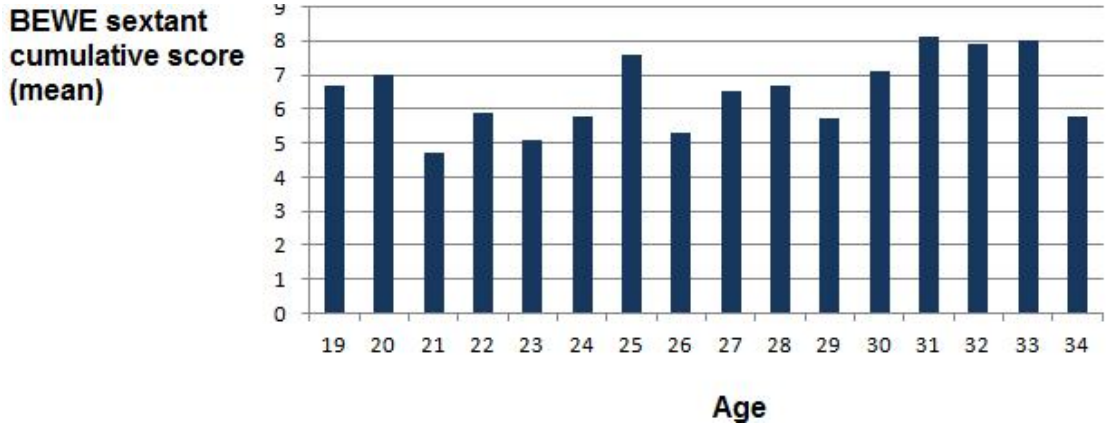


Figure 25 BEWE sextant cumulative score (mean) by age

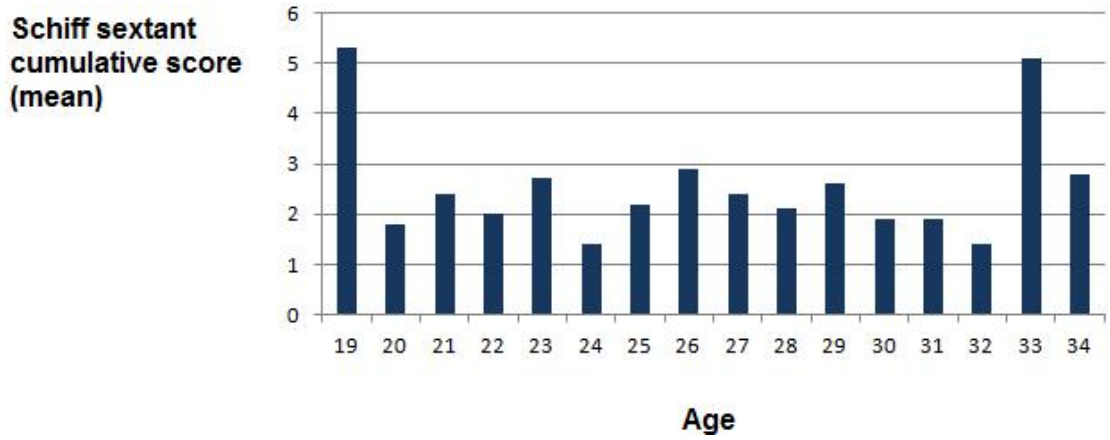


Figure 26 Schiff sextant cumulative score (mean) by age

3.5.4 Tooth wear per tooth

Less than a quarter (21.2%, n=5, 106) of tooth surfaces exhibited tooth wear using the BEWE index (scores 1 and above) and included 24.5% (n=1, 970) buccal tooth surfaces, 40.6% (n=3, 254) occlusal/incisal tooth surfaces and 5.5% (n=440) lingual/palatal tooth surfaces.

Figure 27 shows the percentage of tooth wear (scored using a BEWE score of 1, 2 or 3) on teeth apart from the 3rd molars. Teeth with the most recorded wear were second molars (98.9%), followed by central incisors (70.2%), first molars (57.0%), lateral incisors (57.0%), second premolars (28.4%), canines (24.1%) and finally first premolars (16.4%).

Figure 28 shows the percentage of tooth surfaces (buccal, occlusal/incisal, lingual/palatal) with tooth wear (BEWE 1, 2 or 3). On these graphs, tooth wear is more prevalent on incisal and posterior teeth. However, for occlusal surfaces, the premolar and canine surfaces have similar amounts of wear to incisal and molar teeth (although

wear is mainly BEWE 1). The lingual surfaces had substantially less wear than buccal and occlusal surfaces and wear was often BEWE 1.

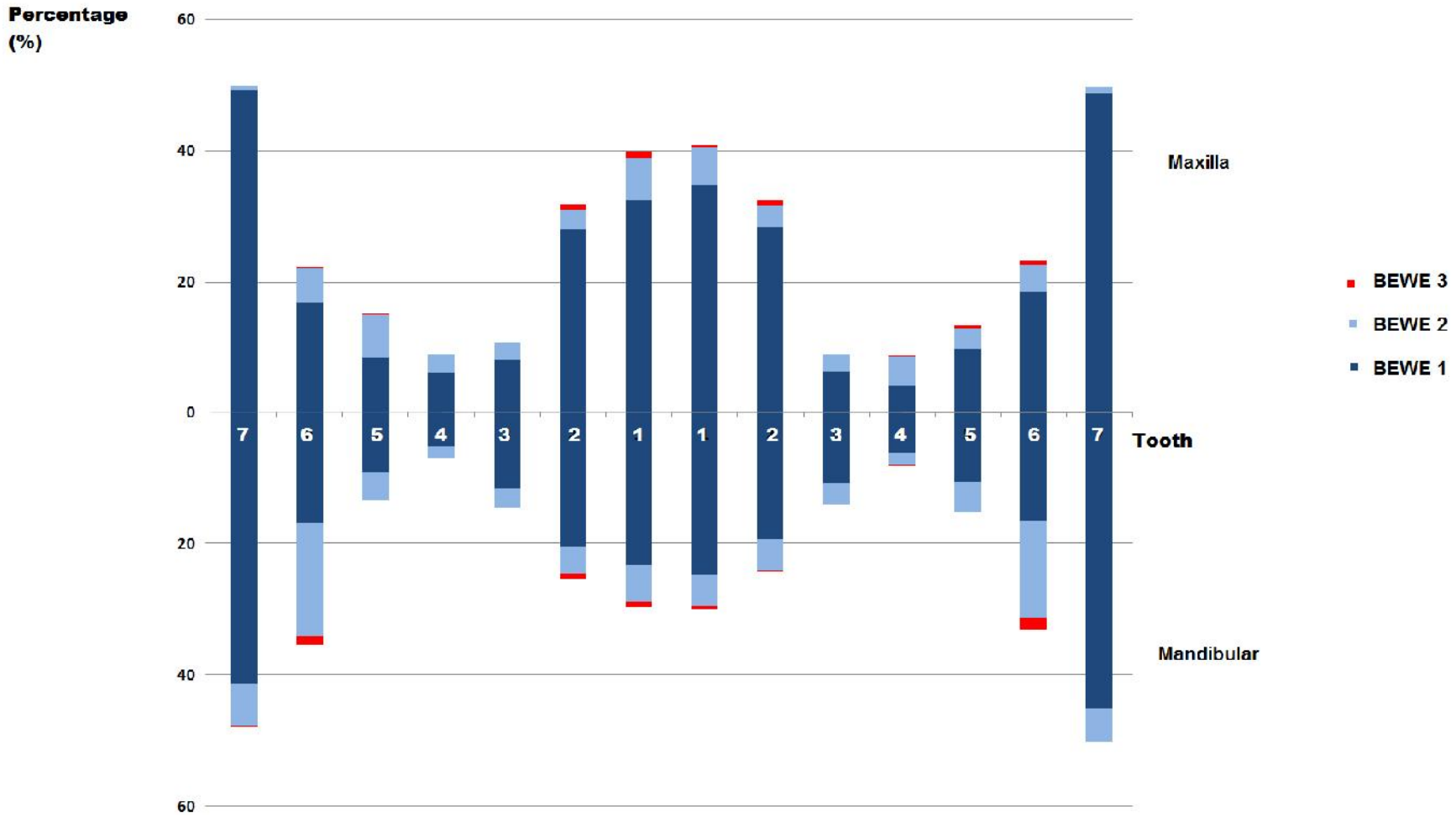


Figure 27 Percentage of tooth wear (BEWE 1, 2 or 3) on teeth 1-7

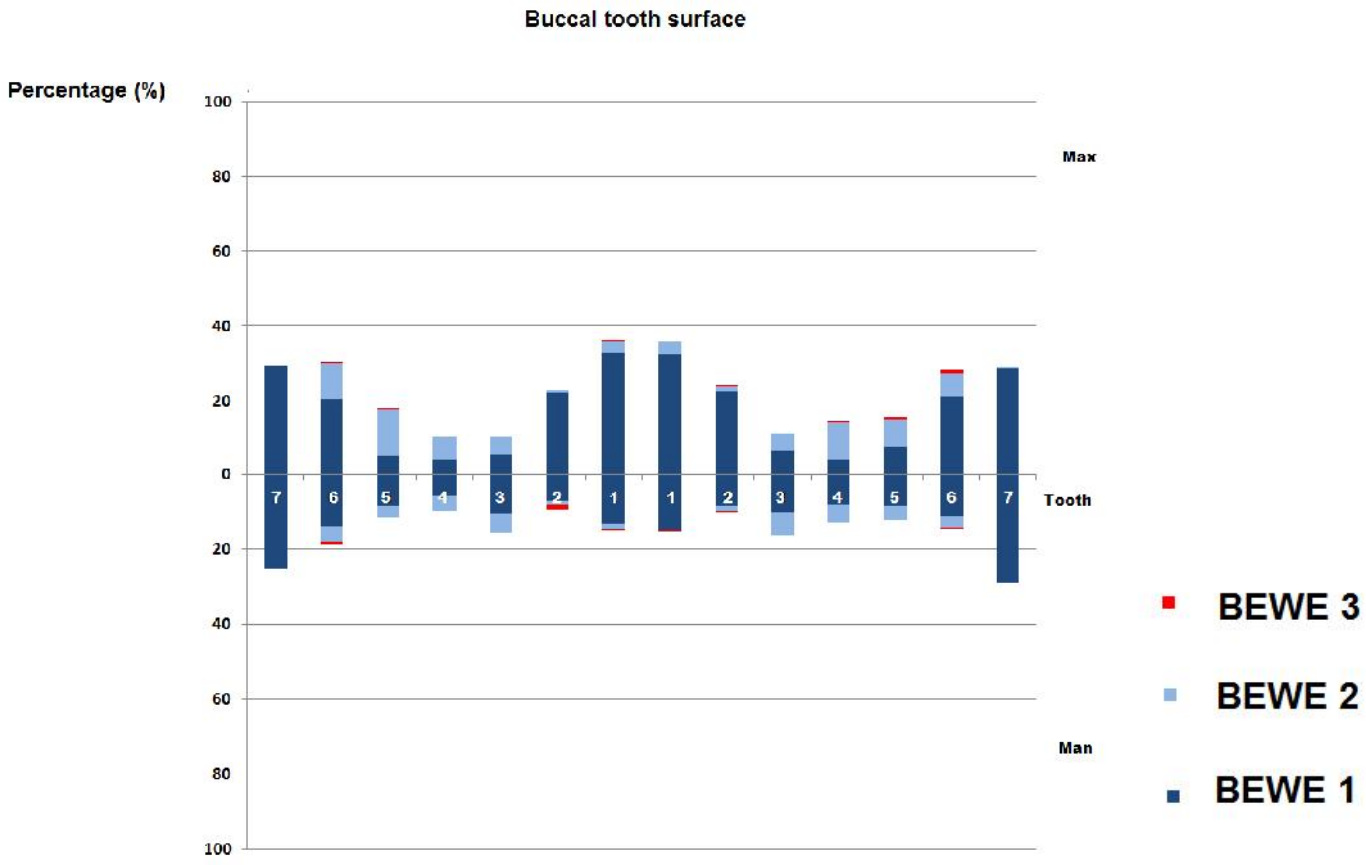


Figure 28 Percentage of tooth surfaces (buccal) with tooth wear (BEWE 1, 2 or 3) on teeth 1-7

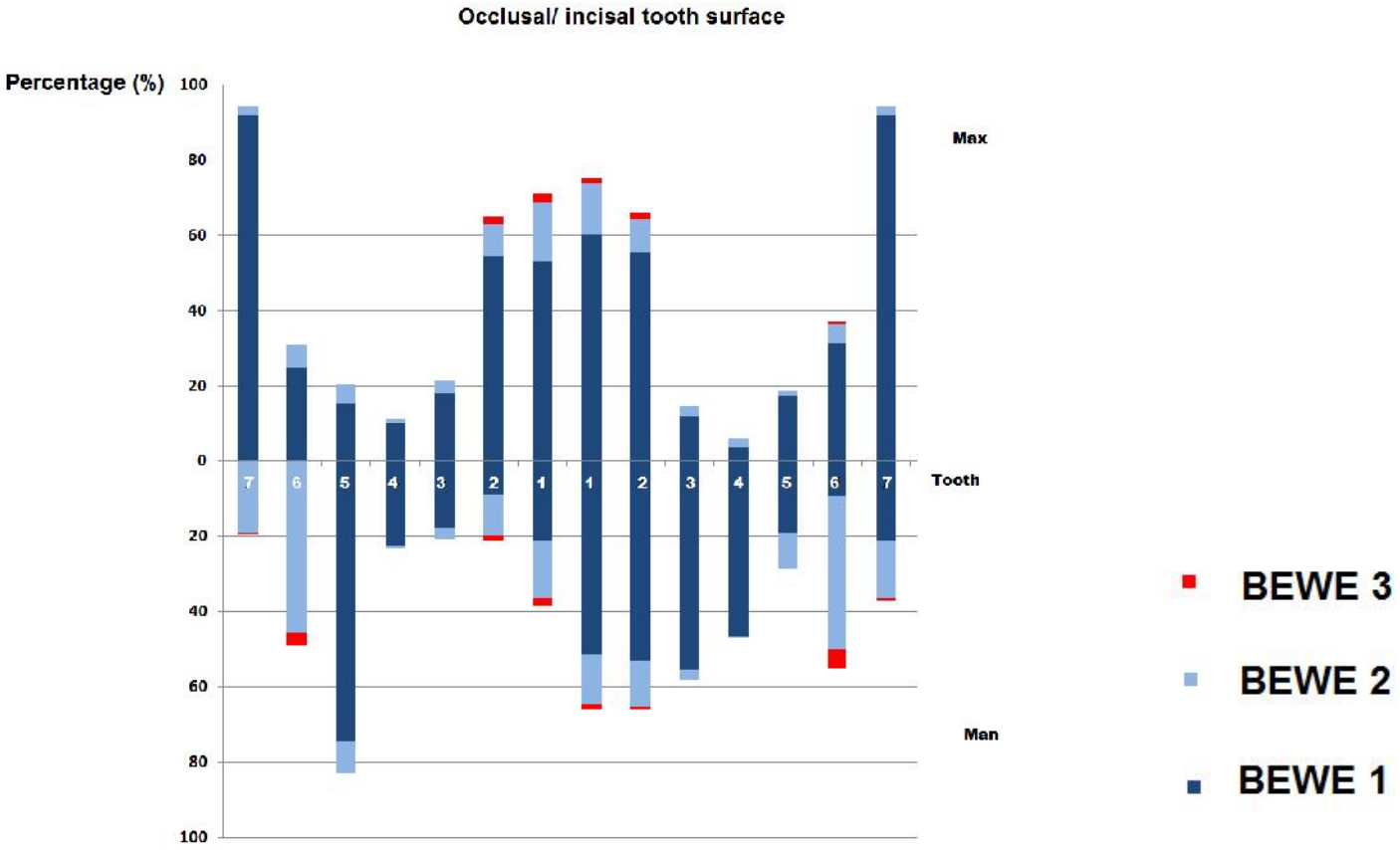


Figure 29 Percentage of tooth surfaces (occlusal/incisal) with tooth wear (BEWE 1, 2 or 3) on teeth 1-7

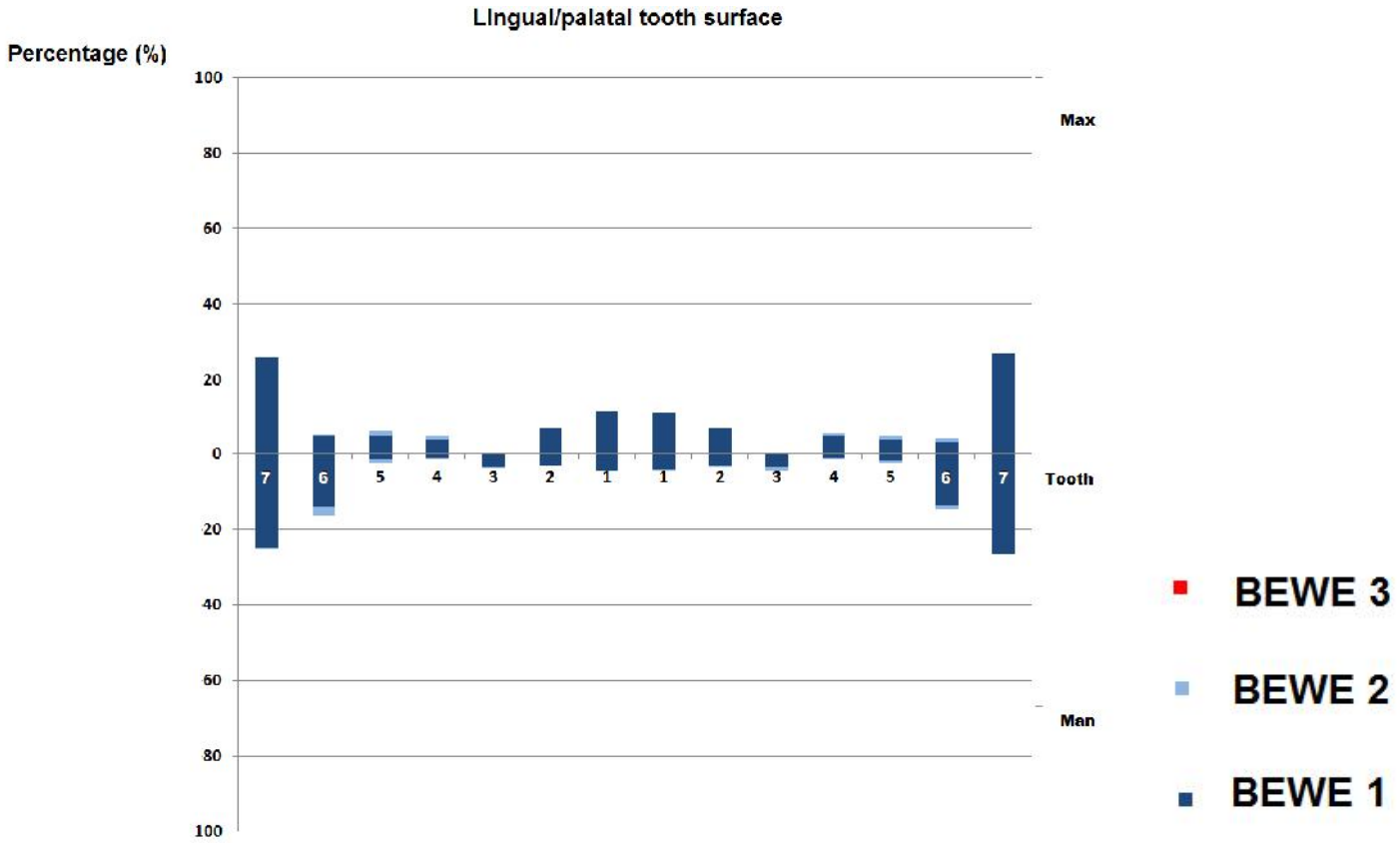


Figure 30 Percentage of tooth surfaces (lingual/palatal) with tooth wear (BEWE 1, 2 or 3) on teeth 1-7

3.5.5 DH per tooth

Among all tooth surfaces examined, 3.4% (n=796) exhibited DH using the Schiff index (score 1 and above) and included 5.5% (n=455) buccal tooth surfaces, 2.4% (n=192) occlusal/incisal tooth surfaces and 1.9% (n=149) lingual/palatal tooth surfaces.

Figure 28 shows the percentage of DH (scored using Schiff 1, 2 or 3) on teeth excluding 2nd and 3rd molars. DH was present on first molars (12.4%), followed by second premolars (7.2%), first premolars (5.8%), central incisors (4.7%), canines (4.6%) and finally lateral incisors (2.7%).

Figure 32 shows the percentage of tooth surfaces (buccal, occlusal/incisal, lingual/palatal) with DH (Schiff 1, 2 or 3) on teeth 1-6. These graphs show that buccal tooth surfaces generally have more DH, although lingual/palatal and occlusal/incisal surfaces demonstrate higher DH prevalence on first molars and first incisors. As shown in section 2.1.3, lingual/palatal surfaces had substantially less DH than buccal and occlusal surfaces and DH was often Schiff 1.

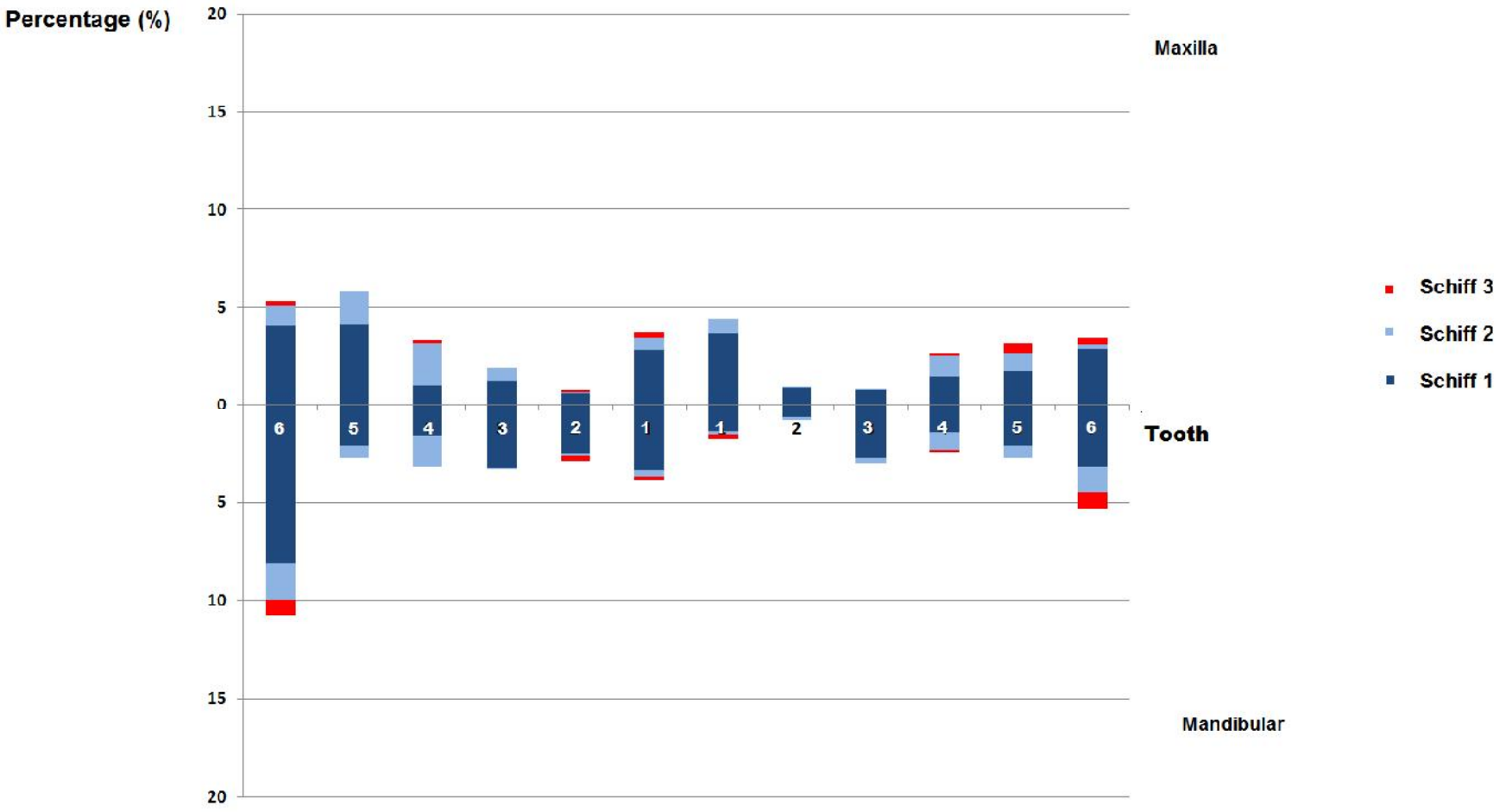


Figure 31 Percentage of DH (Schiff score 1, 2 or 3) on teeth 1-6

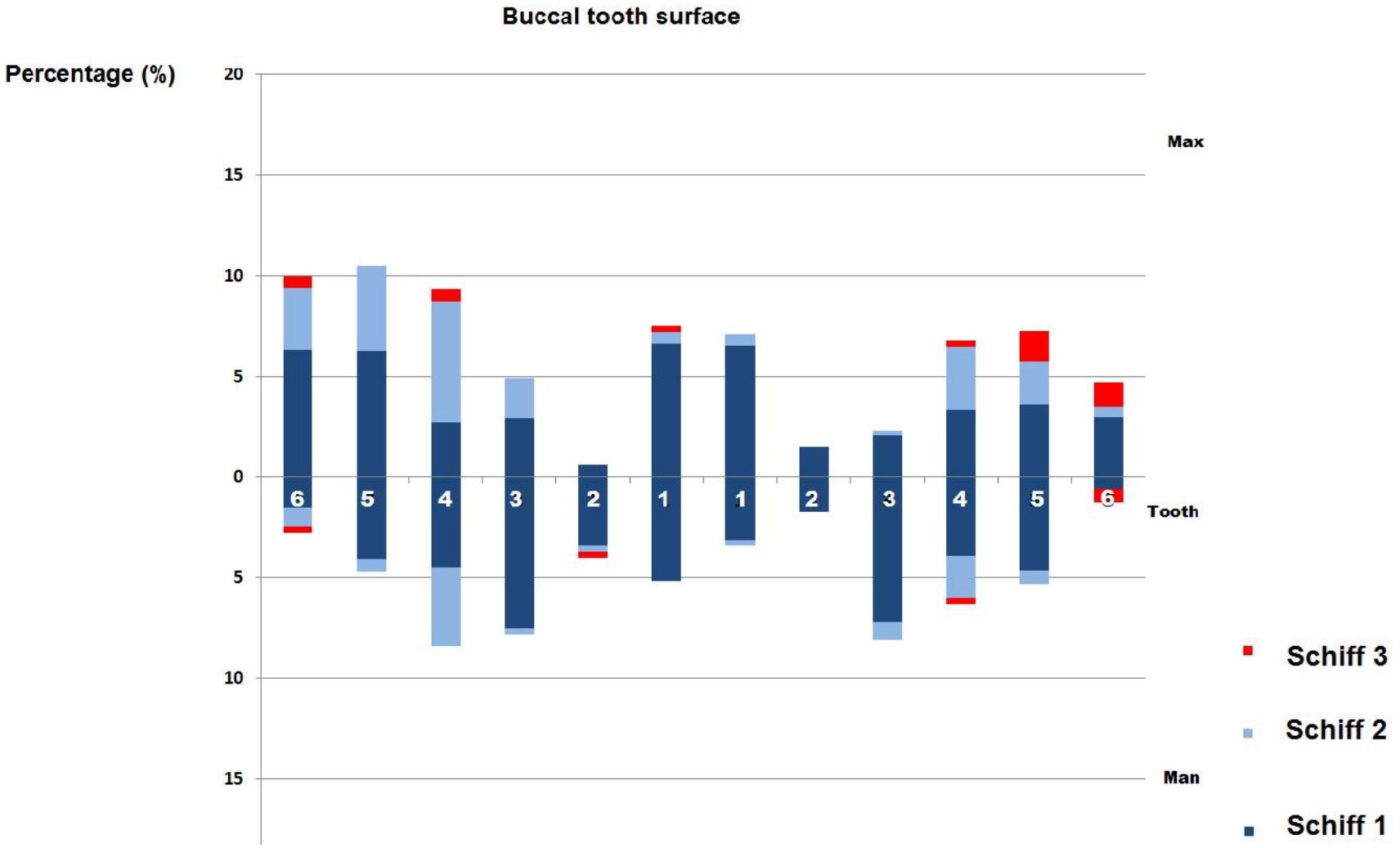


Figure 32 Percentage of tooth surfaces (buccal) with DH (Schiff 1, 2 or 3) on teeth 1-6

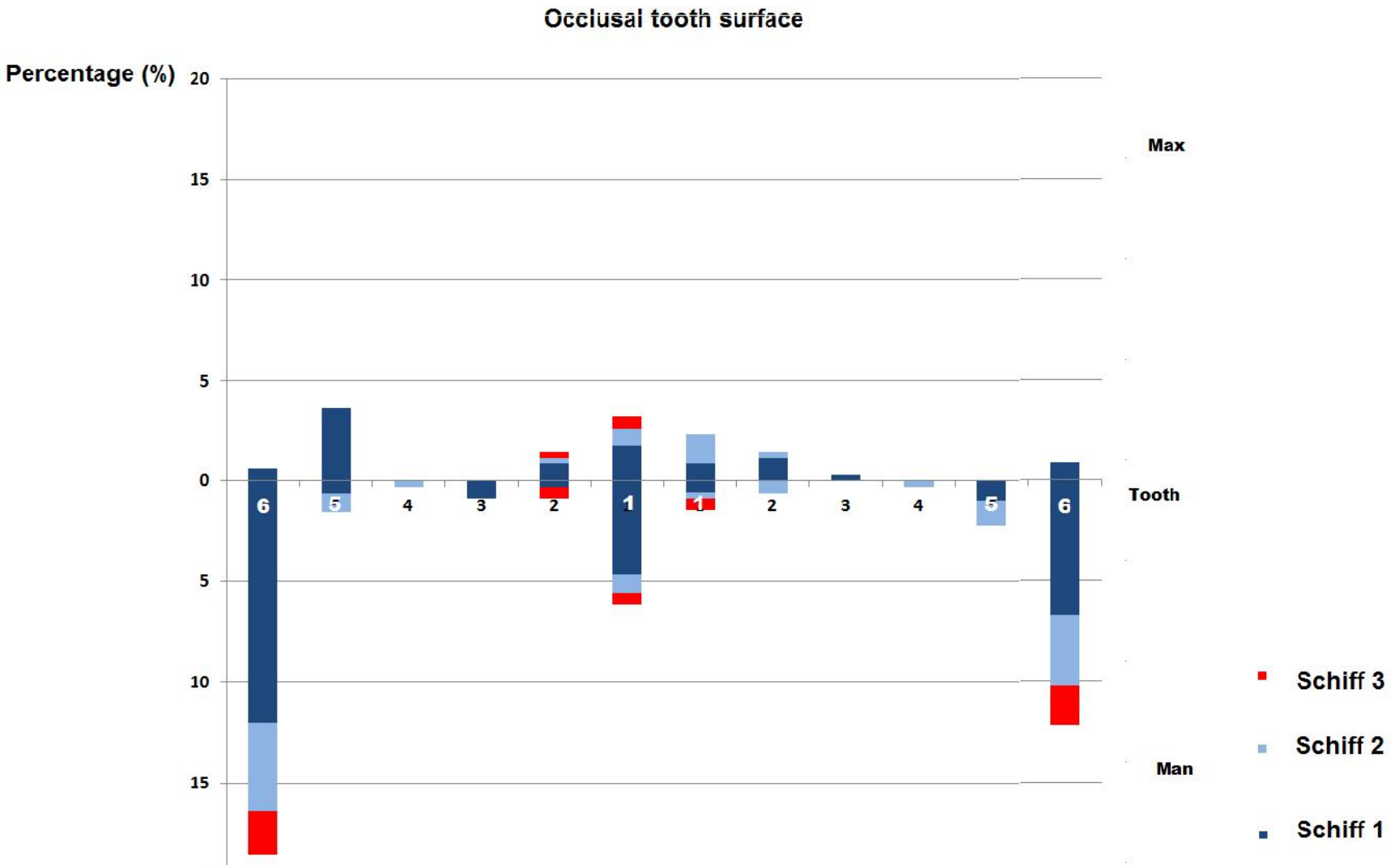


Figure 33 Percentage of tooth surfaces (occlusal/incisal) with DH (Schiff 1, 2 or 3) on teeth 1-6

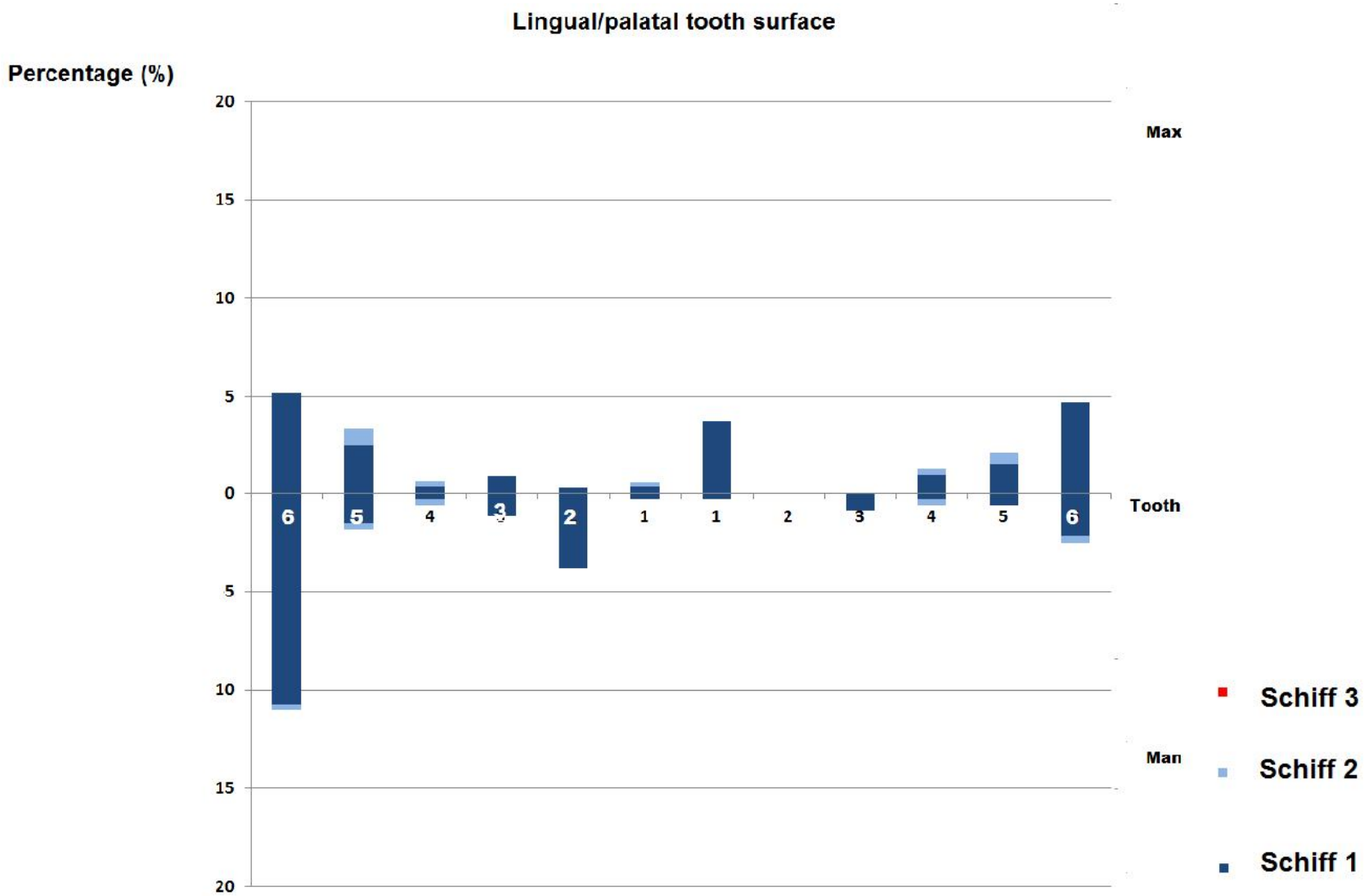


Figure 34 Percentage of tooth surfaces (lingual/palatal) with DH (Schiff 1, 2 or 3) on teeth 1-6

3.5.5.1 Tooth wear and DH per tooth surfaces

Most tooth surfaces (77.7%, n=16, 370) had a score of 0 for Schiff and a score of 0 for BEWE and therefore had no recorded DH or tooth wear respectively. This included 80.2% (n=6, 461) of buccal surfaces, 59.3% (n=4, 750) of occlusal/incisal surfaces and 93.4% (n=7, 500) of lingual/palatal surfaces with no wear or DH.

On 79% (n=19, 033) of all tooth surfaces (n=24, 093), the BEWE score was numerically equivalent to the Schiff score i.e. BEWE 0 and Schiff 0, or BEWE 1 and Schiff 1 etc. The BEWE score also matched the Schiff score on 82.4% (n=6, 036) buccal tooth surfaces, 60.4% (n=4, 840) of occlusal/incisal tooth surfaces and 94.1% (n=7, 552) of lingual/palatal tooth surfaces respectively. Table 23 shows the frequency of all tooth surfaces affected by tooth wear and DH. This table also shows that for tooth surfaces with a BEWE score of 0, 1 and 2 (93.6%, n=20, 992), more tooth surfaces had a lower Schiff score. However, in tooth surfaces with a BEWE score 3 (6.4%, n=92), 49 tooth surfaces (52.7%) had DH recorded using Schiff and were more likely to have a higher Schiff score. No BEWE score of 3 was recorded on lingual surfaces.

Table 23 Frequency of tooth surfaces affected by tooth wear (recorded as BEWE) and DH (recorded as SCHIFF) on all tooth surfaces for teeth 1-6

Total surfaces 100% (n=24, 093)		BEWE Score			
		0	1	2	3
SCHIFF score	0	77.662% (n=18711)	15.054% (n=3627)	3.827% (n=922)	0.195% (n=47)
	1	0.984% (n=237)	0.701% (n=169)	0.627% (n=151)	0.037% (n=9)
	2	0.154% (n=37)	0.071% (n=17)	0.469% (n=113)	0.050% (n=12)
	3	0.008% (n=2)	0.004% (n=1)	0.021% (n=5)	0.137% (n=33)

Table 24 Frequency of tooth surfaces affected by tooth wear (recorded as BEWE) and DH (recorded as SCHIFF) on buccal tooth surfaces for teeth 1-6

Buccal tooth surfaces 100% (n=8, 053)		BEWE Score			
		0	1	2	3
SCHIFF score	0	80.231% (n=6461)	11.275% (n=908)	2,856% (n=230)	0.112% (n=9)
	1	1.751% (n=141)	1.267% (n=102)	0.807% (n=65)	0.025% (n=2)
	2	0.459% (n=37)	0.174% (n=14)	0.770% (n=62)	0.037% (n=3)
	3	0.025% (n=2)	0.000% (n=0)	0.062% (n=5)	0.149% (n=12)

Table 25 Frequency of tooth surfaces affected by tooth wear (recorded as BEWE) and DH (recorded as SCHIFF) on occlusal/incisal tooth surfaces for teeth 1-6

Occlusal/incisal tooth surfaces 100% (n=8, 014)		BEWE Score			
		0	1	2	3
SCHIFF score	0	59.271% (n=4750)	29.523% (n=2366)	8.335% (n=668)	0.474% (n=38)
	1	0.125% (n=10)	0.324% (n=26)	0.961% (n=77)	0.087% (n=7)
	2	0.000% (n=0)	0.000% (n=0)	0.512% (n=41)	0.112% (n=9)
	3	0.000% (n=0)	0.012% (n=1)	0.000% (n=0)	0.262% (n=21)

Table 26 Frequency of tooth surfaces affected by tooth wear (recorded as BEWE) and DH (recorded as SCHIFF) on lingual/palatal tooth surfaces for teeth 1-6

Lingual/palatal tooth surfaces 100% (n=8, 026)		BEWE Score			
		0	1	2	3
SCHIFF score	0	93.446% (n=7500)	4.398% (n=353)	0.299% (n=24)	0.000% (n=0)
	1	1.072% (n=86)	0.511% (n=41)	0.112% (n=9)	0.000% (n=0)
	2	0.000% (n=0)	0.037% (n=3)	0.125% (n=10)	0.000% (n=0)
	3	0.000% (n=0)	0.000% (n=0)	0.000% (n=0)	0.000% (n=0)

3.5.5.2 Brushing habits

Subjects reported to brush once (6.9%, n=24), twice (82.6%, n=289), three (9.4%, n=33) or four (0.9%, n=3) times per day and the remainder (0.2%, n=1) did not answer. They used a manual toothbrush (62.3%, n=218), an electric toothbrush (36.3%, n=127) or no toothbrush (1.1%, n=4) and the remainder (0.2%, n=1) did not answer. Subjects reported their tooth brushing motions as various (66.3%, n=232), circular (18.9%, n=66), horizontal or 'back and forth' movement (6.9%, n=24), vertical or 'up and down' movements (6.0%, n=21) or they were not sure (1.4%, n=5) and the remainder did not answer (0.5%, n=2). Subjects were mostly right-handed (87.2%, n=305) or left-handed (10.9%, n=38) and the remainder (1.9%, n=7) did not answer.

Figure 35 shows the percentage of tooth brushing before breakfast, after breakfast, after lunch and after dinner. Most (91.7% n=219) of all subjects had reported brushing their teeth "often" or "occasionally" before breakfast. In addition, 62.8% (n=219) of all subjects reported brushing "often" or "occasionally" after breakfast. Just over half of all subjects (57.4%, n=201) also brushed "often" or "occasionally" after lunch and most (96.3%, n=337) also brushed "often" or "occasionally" after dinner. Of the subjects who brushed after breakfast, 3.7% (n=13) reported brushing immediately and 96.0% (n=315) reported brushing up to two hours after breakfast. The mean delay between breakfast and brushing was 17.76 minutes, (SD 19.20, SE 1.1).

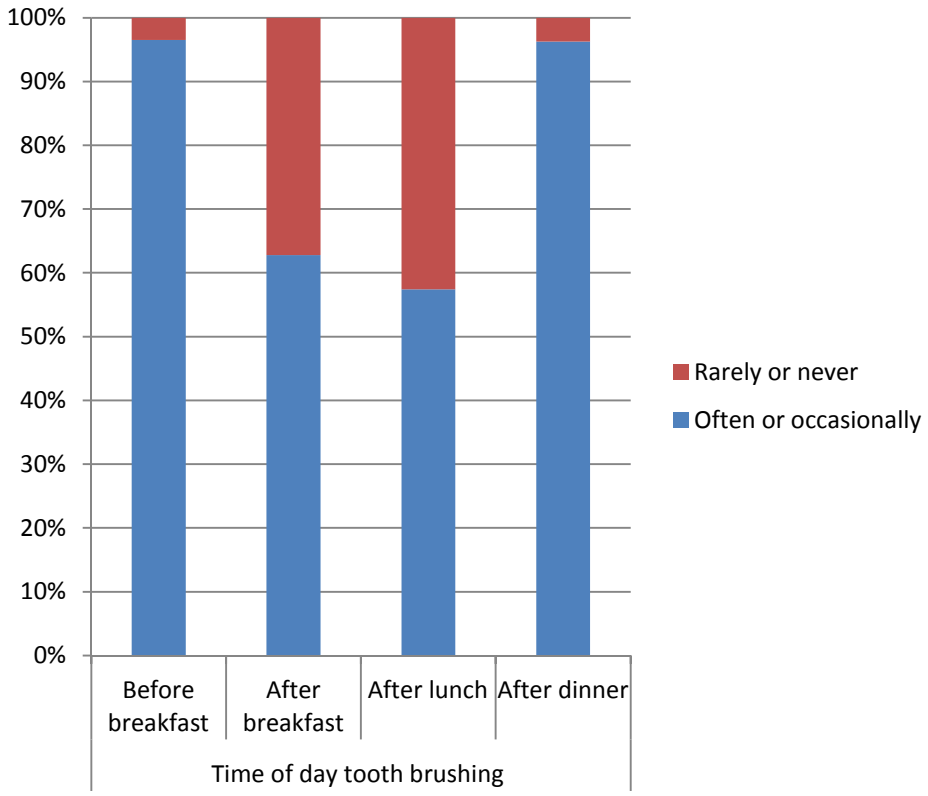


Figure 35 Percentage of tooth brushing habits during the day

Figure 36 shows that subjects who reported brushing their teeth soon after breakfast (within 20 minutes) were more likely to have experienced DH “often” over the previous 12 months.

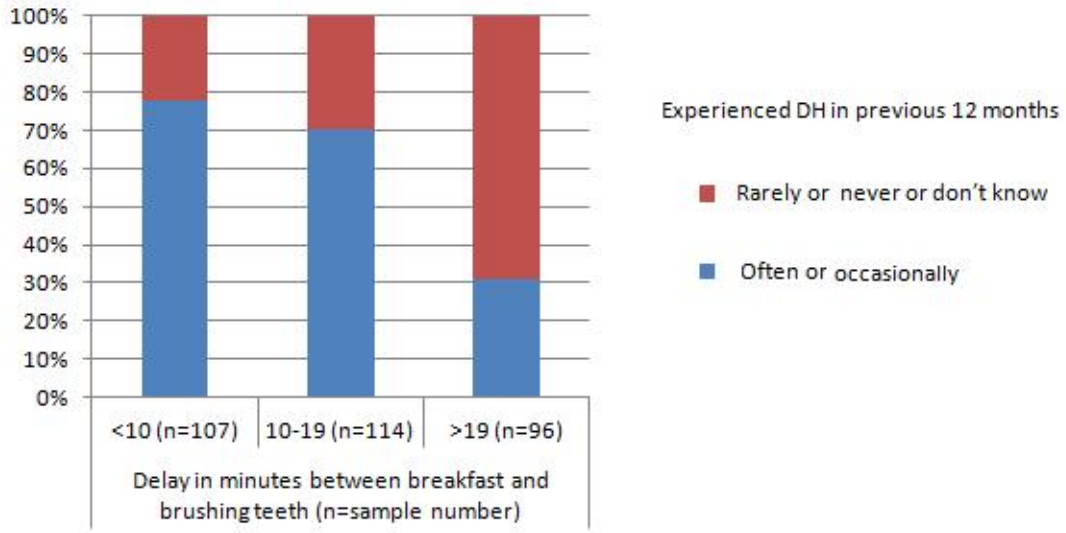


Figure 36 Delay between breakfast and brushing teeth for subjects who have experienced DH in the past twelve months (n=317)

3.5.5.3 Life events

Figure 37 shows the prevalence of DH over the previous 12 months for those subjects who had suffered various life events, including events indicative of anxiety or depression. Subjects who experienced each lifestyle factor were more likely to have experienced DH in the previous 12 months ($p<0.05$). Subjects who reported they had suffered from repeated vomiting or heartburn, reflux and regurgitation were more likely to report toothache due to DH “often” or “occasionally” over the same time period. In addition, subjects who reported they had “often” or “occasionally” experienced difficulties with eating food due to teeth or mouth problems, felt embarrassed because of the appearance of their teeth, felt tense because of teeth or mouth problems or avoided conversation because of the appearance of their teeth or dentures in the past twelve months, were more likely to have report toothache due to DH “often” or “occasionally” in the same time period.

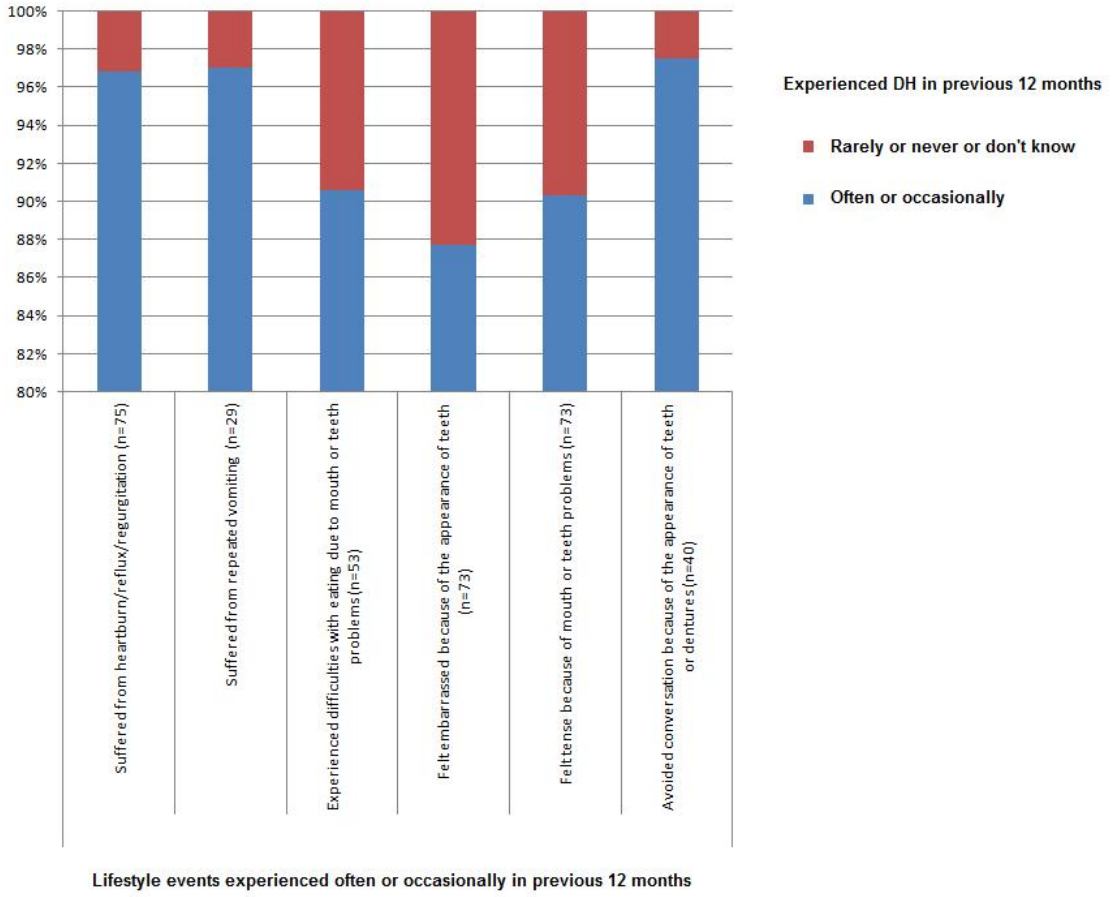


Figure 37 Prevalence of DH over the past 12 months for various life events

3.5.5.4 Current tooth sensitivity

Subjects who currently reported DH in the questionnaire (44.3%, n=155), indicated this was “often” or “occasionally” due to tooth brushing (78.2%, n=122), cold drinks or ice (64.1%, n=100), cold weather (62.8%, n=98), touch (51.2%, n=80), sweet (35.3%, n=55), hot water (32.9%, n=51) or other stimuli (20.5%, n=31). Figure 38 shows the causes of DH recorded by patients who were currently suffering from DH.

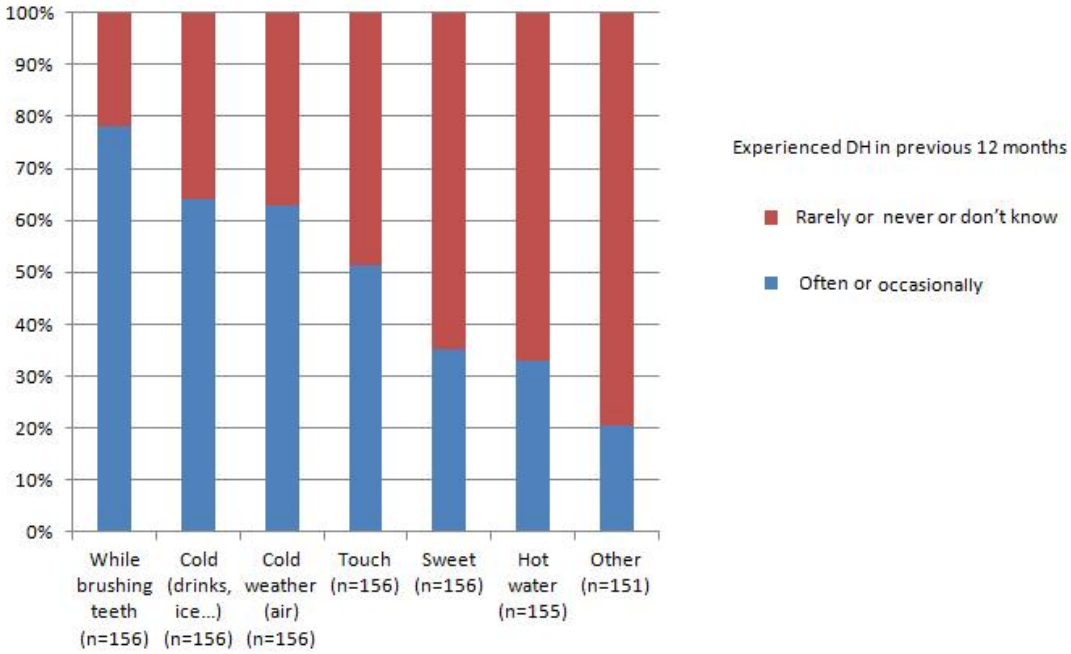


Figure 38 Stimuli required to elicit DH for those subjects who currently report tooth sensitivity (n=155)

Of those subjects who reported current DH, over half (57%, n=89) recorded this as "very important" or "important" with the remainder classifying this as "somewhat important", of "little importance" or "not important". In addition, the majority (70.5%, n=110) recorded that DH had lasted between 1 and 5 years.

3.5.5.5 Acidic consumables

Subjects reported that they consumed acidic food and drinks "often" (21.7%, n=76), "occasionally" (42.6%, n=149),"rarely" (24.6%, n=86), "never" (10.6%, n=37) or "did not know" (0.6%, n=2). Figure 39 shows the frequency of acid consumption per day for subjects who currently reported having DH. Subjects who currently report DH were more likely to consume more acidic beverages.

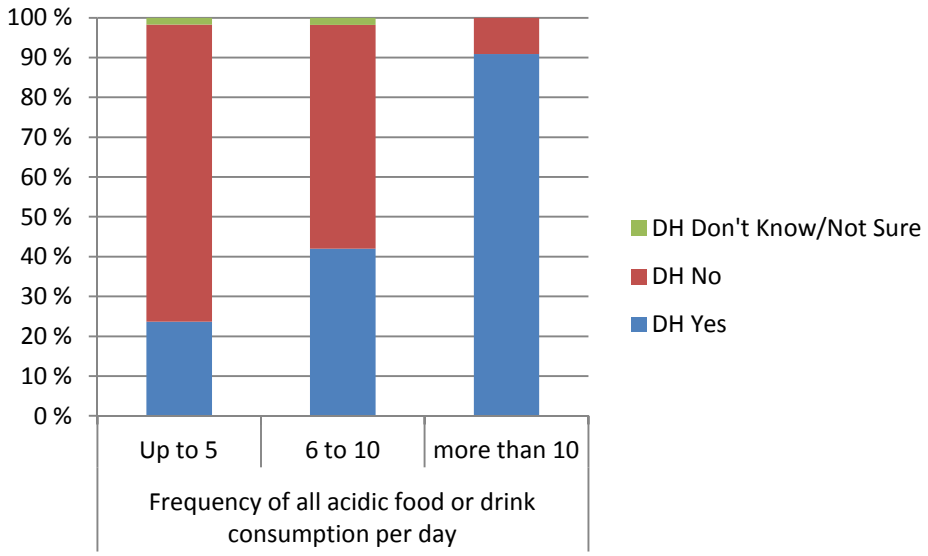


Figure 39 Frequency of acid consumption per day for subjects with and without DH

Among subjects who reported consuming fresh fruit, isotonic drinks, soft drinks, cheeses and fruit or vegetable juice “often”, 71% (n=87), 68% (n=61), 53% (n=40), 43% (n=33) and 70.9% (n=73) reported consuming these twice or more per day respectively. Of the subjects who reported consuming fruit juice, isotonic drinks, soft drinks, cheese and fruit or vegetable juice twice or more per day, most (81.6%, n=71; 90.2%, n=55; 72.5%, n=29; 57.6%, n=19; 86.3%, n=63 respectively) reported that they had current DH.

In the remainder of subjects who consumed fresh fruit, isotonic drinks, soft drinks, cheeses and fruit or vegetable juice “often”, 28.7%, (n=35), 32.2%, (n=29), 47.4%, (n=36), 57.1% (n=44) and 29.1% (n=30) reported consuming these once per day or less respectively. Amongst the subjects who consumed fresh fruit, isotonic drinks, soft drinks, cheeses and fruit or vegetable juice once per day or less, the majority did not report current DH (54.2%, n=19; 72.4%, n=21; 55.6% (n=20); 52.3%, n=23; 70.9%, n=73). Figure 40 shows whether subjects reported having current DH for consumption of various acidic foods and drinks once or less per day and two or more times per day.

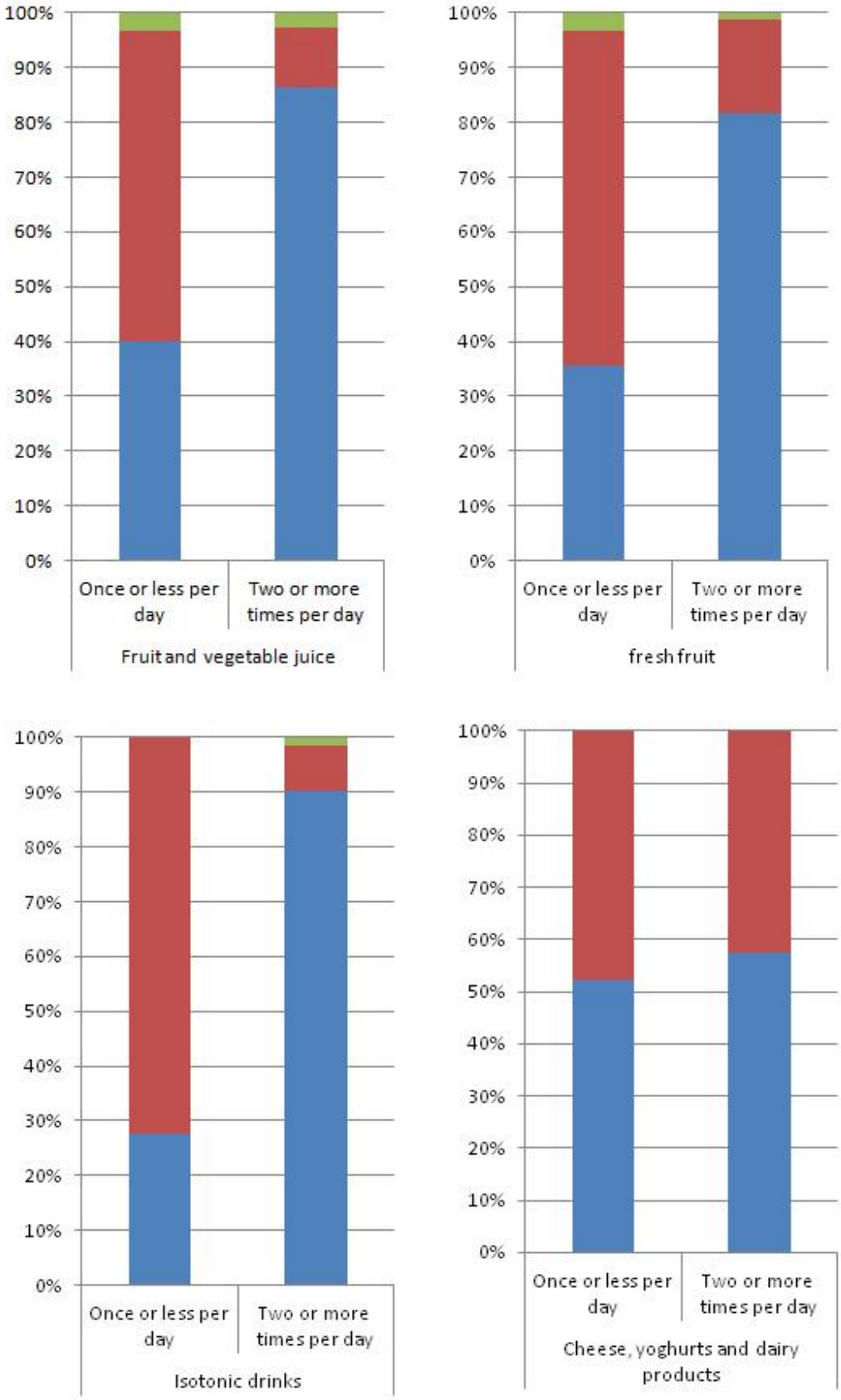


Figure 35 continued on next page.

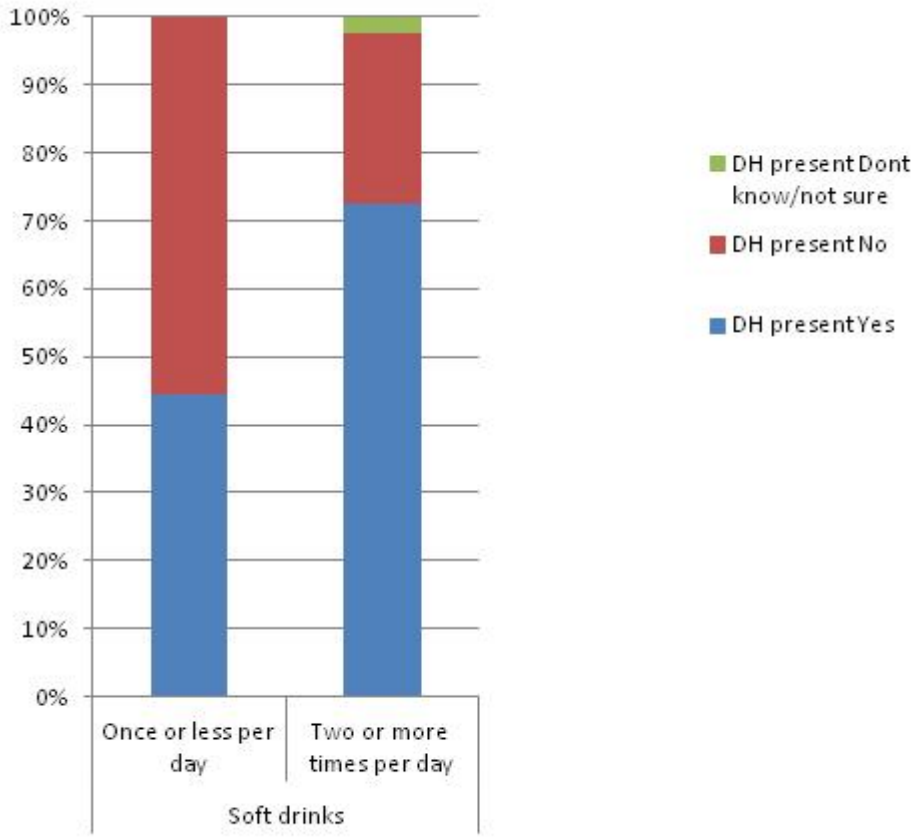


Figure 40 Frequency of acid consumption per day for subjects who consume acids often and are with or without DH

Most (99%, n=345) of all subjects provided information on when they last consumed an acidic food or drink prior to their clinical examination. Subjects who had DH recorded clinically on at least one tooth surface were more likely to report that they had consumed an acidic food or drink within 60 minutes of their examination (87.2%, n=166). Subjects who did not have DH recorded clinically were more likely to report that they had consumed an acidic food or drink more than 60 minutes prior to their examination (84.7%, n=166). This is shown in Table 27.

Table 27 Time since last acid consumed and whether DH is recorded clinically using Schiff (n=345)

Time since last acid consumed (minutes)	Negative DH	Positive DH
≤60	15.3% (n=30)	87.2% (n=130)
>60	84.7% (n=166)	12.8% (n=19)
Total	100% (n=196)	100% (n=149)

3.5.5.6 Recession and DH

The total number of buccal and palatal tooth surfaces (n=16, 553) affected by gingival recession was 13.1% (n=2, 164). Of the tooth surfaces with gingival recession, 12.7% (n=275) had DH (recorded using Schiff) and were more likely to have a lower Schiff score. In addition, subjects without gingival recession were also likely to have a lower Schiff score. This is shown in Figure 41.

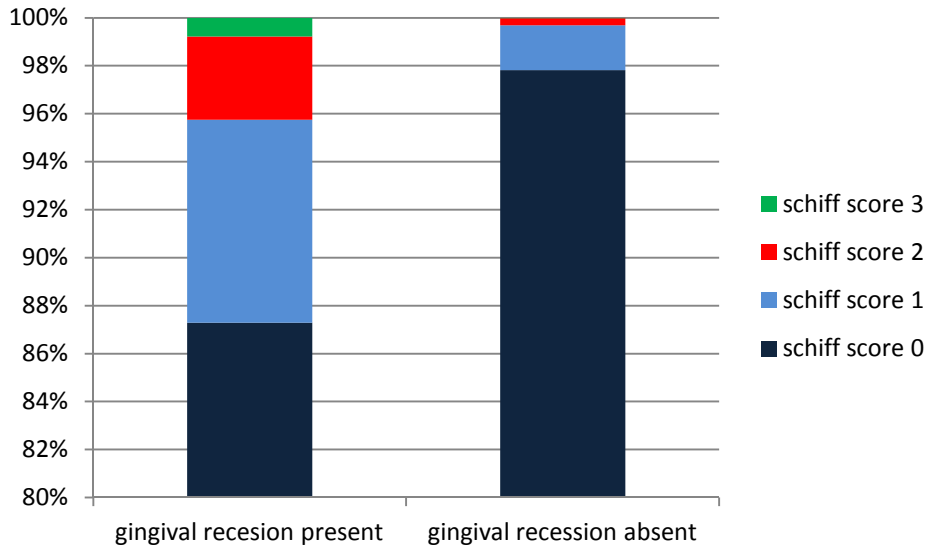


Figure 41 Percentage of buccal and palatal tooth surfaces with DH recorded using Schiff on teeth 1-6 with and without gingival recession

3.5.6 BEWE group 0

BEWE group 0 represents those subjects in which there was no recorded tooth wear on any tooth surface (9.1%, n=32). The mean age was 25.7 (SD 3.4) years. Most

(62.5%) were female. The majority were seen in primary care sites (68.7%, n=22) and the remainder in secondary care (31.3%, n=10). Their education was to 16-19 (34.4%, n=11), 20+ (37.5%, n=12) or they were still studying (28.1%, n=9). The largest occupation groups were white collar (37.5%, n=12), self-employed (28.1%, n=9) or student (18.8%, n=6).

The majority brushed twice a day (78.1%, n=25) using a manual (71.9%, n=23) or electric toothbrush (28.1%, n=9). Their brushing motion was often various (68.8%, n=22). They brushed “often” or “occasionally” before breakfast (62.5%, n=20), but occasionally, “rarely” or “never” after breakfast (93.8%, n=30) and after lunch (78.1%, n=25). Most brushed often after dinner (71.9%, n=23). In 62% (n=20), the delay between having breakfast and brushing was 15 minutes or less.

Most (68.7%, n=22) had reported experiencing DH “rarely” or “never” in the previous 12 months. The majority had “rarely” or “never” had indigestion/heartburn (87.5%, n=28), vomiting or had eating problems (93.8%, n=30). Most had never felt embarrassed (71.9%, n=23), tense (75%, n=24) or avoided conversation (81.3%, n=26) in the previous 12 months due to mouth or teeth problems. Most had “rarely” or “never” reported snoring (84.4%, n=21), took sleeping medications (84.4%, n=27) or smoked (87.5%, n=28) or chewed gum (78.1%, n=25).

Most reported not having DH “now” (90.6%, n=115). Of those who reported DH (23.5%, n=36), it had often lasted less than a year (90.6%, n=29) and was generally of “no importance” (90.6%, n=29).

The majority occasionally or rarely consumed acidic food (81.3%, n=26). In addition, the time they had last consumed an acidic beverage was often more than 15 minutes

prior to their clinical examination (93.7%, n=30). Most (68.8%, n=22) had attended for a check-up, examination or cleaning appointment. Most played a sport between 1 and 4 times per week (46.9%, n=117) or were not sure or played less often (28.2%, n=9). Most (87.5%, n=28) used fluoride toothpaste.

3.5.7 BEWE group 1

BEWE group 1 represents those subjects in which there was initial loss of surface characteristic on at least one tooth surface (43.7%, n=153). The mean age was 26.3 (SD 3.5) years. Most were female (54.2%, n=83) and the majority were seen in primary care sites (58.0%, n=89) with the remainder in secondary care (42.0%, n=64). Their education was to 16-19 (33.3%, n=51), 20+ (35.9%, n=55) or they were still studying (28.8%, n=44). The largest occupation groups were student (28.1%, n=43), white collar (24.8%, n=38) or self-employed (18.3%, n=28).

The majority brushed twice a day (78.4%, n=120) using a manual (66%, n=101) or electric toothbrush (32.7%, n=50). Their brushing motion was often various (64.7%, n=99). They brushed often or occasionally before breakfast (93.4%, n=143), after breakfast (62.1%, n=95), after lunch (56.9%, n=87), and after dinner (96.8%, n=148). In 58.8% (n=90), the delay between having breakfast and brushing was 15 minutes or less. Most had “rarely” or “never” reported snoring (64.1%, n=98), took sleeping medications (88.3%, n=135), smoked (91.5%, n=140) or chewed gum (70.6%, n=108).

Less than half had reported experiencing DH “often” or “occasionally” in the previous 12 months (48.4%, n=74). The majority had “rarely” or “never” had indigestion/heartburn (88.9%, n=136), vomiting (90.8%, n=139), had eating problems (90.2%, n=138), felt embarrassed (86.3%, n=132), tense (85%, n=130) or avoided

conversation (94.1%, n=144) in the previous 12 months due to mouth or teeth problems.

Most reported not having DH “now” (75.2%, n=115). Of those who reported DH (23.5%, n=36), it had “often” lasted up to 5 years (91%, n=33) and was generally of “little or some importance” (64%, n=23).

The majority “occasionally” or “rarely” consumed acidic food (71.9%, n=110). In addition, the time they had last consumed an acidic beverage was often more than 15 minutes prior to their clinical examination (86.8%, n=78). Most (62.1%, n=95) had attended for a check-up, examination or cleaning appointment. Most played a sport between 1 and 3 times per month and up to 3 or 4 times per week (76.5%, n=117). Most (90.8%, n=139) used fluoride toothpaste.

3.5.8 BEWE group 2

BEWE group 2 represents those subjects in which a distinct tooth defect occurred on less than 50% of a tooth surface (36.9%, n=129). The mean age was 26.4 (SD 3.7) years. Most were female (51.9%, n=67). The majority were seen in primary care sites (62.0%, n=80) and the remainder in secondary care (38.0%, n=49). Their education was to 16-19 (35.7%, n=46), 20+ (35.7%, n=46) or they were still studying (25.6%, n=33). The largest occupation groups (>10%) were white collar (29.5%, n=38), student (24.0%, n=31), self-employed (18.6%, n=24) or unemployed (12.4% (n=31).

The majority brushed twice a day (85.3%, n=110) using a manual (55%, n=71) or electric toothbrush (43.4%, n=56). Their brushing motion was often various (66.7%,

n=86). They brushed “often” or “occasionally” before breakfast (90%, n=116), and just over half (58%, n=76) brushed after breakfast and lunch. Most (83%, n=107) brushed after dinner. In 69.1% (n=89), the delay between having breakfast and brushing was 15 minutes or less.

They had experienced DH “often” or “occasionally” in the previous 12 months (74.4%, n=96). The majority had “rarely” or “never” had indigestion/heartburn (70.6%, n=91), vomiting (92.3%, n=119), had eating problems (78.3%, n=101), felt embarrassed (72.1%, n=93), tense (71.3%, n=92) or avoided conversation (83.6%, n=107) in the past 12 months due to mouth or teeth problems.

Most of them reported DH “now” (65.9%, n=31) and this was mostly caused “often” or “occasionally” by brushing (52%, n=67) and secondly due to cold weather (43.5%) or cold drinks (42.6%, n=55). Most had suffered sensitivity for less than a year or up to 5 years (75.2%, n=97) and considered it of some importance (15.5%, n=20), important (26.4%, n=34) or very important (17.8%, n=23). Most had “rarely” or “never” taken sleeping medications (79.1%, n=102) or smoked (82.9%, n=107) or chewed gum (62.1%, n=80).

The majority reported “often” or “occasionally” consuming acidic food (76%, n=98). However, the time they had last consumed an acidic beverage was often more than 15 minutes prior to their clinical examination (61.2%, n=78) and of these just over half (55%, n=71) had attended for a check-up, examination or cleaning appointment. Most played a sport between 1 and 3 times per month and up to 5 times per week (86%, n=111). Most (96.1%, n=124) used fluoride toothpaste.

3.5.9 BEWE group 3

BEWE group 3 represents those subjects with the most severe tooth wear in which wear was found on more than 50% of a tooth surface (10.3%, n=36). The mean age was 26.1 (SD 3.2) years. Most were female (75%, n=27). The majority were seen in primary care sites (64.0%, n=23) and the remainder in secondary care (22.0%, n=8). Their education was to 16-19 (33.3%, n=12), 20+ (33.3%, n=12) or they were still studying (30.6%, n=11). The largest occupation groups (>10%) were house persons (27.8%, n=10), student (22.2%, n=8), white collar (19.4%, n=7), manual (11.1%, n=4), or unemployed (11.1% (n=4).

The majority (94.4%, n=34) brushed twice per day using a manual (63.9%, n=23) or electric (33.3%, n=12) toothbrush. Their brushing motion was often various (69.4%, n=25). They brushed often or occasionally before breakfast and after breakfast (86%, n=31). In 83% (n=30), the delay between having breakfast and brushing was 15 minutes or less.

They had experienced DH “often” or “occasionally” in the previous 12 months (80.6%, n=29). The majority had “rarely” or “never” had vomiting (83.4%, n=30), had eating problems (66.6%, n=24) or avoided conversation (72.2%, n=26) or felt tense (61.1%) in the past 12 months due to mouth or teeth problems. Just over half had not had indigestion/heartburn (55.6%, n=20) or felt embarrassed (52.8%, n=19) due to mouth or teeth problems.

Most of them reported DH “now” (86.1%, n=31) and this was mostly caused often or occasionally by brushing (80.5%, n=29) and secondly due to cold drinks (55.6%, n=20). Most had suffered sensitivity for 2-5 years (52.8%, n=19) or 1-2 years (19.4%, n=7)

and considered it of some importance (25%, n=9), important (25%, n=9) or very important (27.8%, n=10). Most had “rarely” or “never” taken sleeping medications (69.4%, n=25) or smoked (61.1%, n=22).

The majority reported “often” or “occasionally” consuming acidic food (86.2%, n=31) and the time they had last had an acidic beverage was 15 minutes or less prior to their clinical examination (69.4%, n=25).

Most had attended a dentist within the past year (69.4%, n=25) and of these 61.1%, n=22 had attended for a check-up, examination or cleaning appointment. Generally, they reported playing sport 4 or more times a week (66.6%, n=24). Most (88.9%, n=32) used fluoride toothpaste.

3.5.9.1 Side of mouth brushed

The majority of the variables showed a skewed distribution and are described using mean, standard deviation, median and inter-quartile range. Left- and right-handed groups were compared using Mann-Whitney-u tests. Comparison of left and right sides within subject were compared using Wilcoxon matched-pairs, signed-ranks tests. This is shown in appendix 7.3. None of the variables were statistically significant for side of mouth brushed.

Similar analyses were repeated for maxilla and mandible sites although no statistically significant differences were observed.

3.5.10 Relationship between Schiff, BEWE and aetiologies

3.5.10.1 Univariate analyses

A univariate analysis was used to measure the strength of association between the Schiff and BEWE sextant cumulative scores and each of the exposure factors respectively on the whole data per subject, without taking account of other potential confounding factors. Assuming that the variable was either ordinal or quantitative and continuous, Spearman rank correlation coefficient and p value were calculated. Assuming that the variable was categorically grouped, then p value for ANOVAs was calculated. These are shown for all variables for the Schiff and BEWE sextant cumulative scores in appendices 7.4 and 7.5 and the factors with significant associations have been highlighted. Statistically significant associations ($p<0.05$) occurred between Schiff and BEWE sextant cumulative scores recorded from the clinical examination and the following exposure factors respectively from the questionnaire:

- Subject gender (male/female)
- Location of subjects practice (metropolitan, rural, town)
- Whether the subject brushes after breakfast (often, occasionally, rarely, never)
- In the past twelve months has the subject often, occasionally, rarely or never;
    - Experienced tooth ache due to sensitive teeth,
    - Suffered from heartburn/reflux/regurgitation,
    - Eating problems,
    - Embarrassed because of the appearance of teeth,
    - Felt tense because of mouth or teeth problems,
    - Avoided conversation because of the appearance of teeth,
- If the subject currently suffers from tooth sensitivity (yes/no),

- Experience sensitive teeth due to brushing teeth, cold weather, touch, hot water, sweet, cold (drinks, ice etc.) and others (often, occasionally, rarely, never),
- Length of time sensitivity has occurred for (less than a year/ 1-2 years/5 or more years/don't know/never),
- How important the pain intensity of sensitive teeth is (Not important, little importance, some importance, important, very important, don't know),
- Snoring frequency, Sleep medications/antidepressants, Smoking, Chewing gum and how often acidic foods or drinks consumed (often, occasionally, rarely, never),
- If fresh fruit, fruit and vegetable juice, energy drinks/isotonic drinks or soft drinks are consumed often, how many times these are consumed,
- How often exercise or play sport (5 times a week or more, 3 to 4 times a week, 1 to 2 times a week, 1 to 3 times a month, don't know, less often, never),
- Time since last consumed fresh fruit, fruit or vegetable juice, isotonic/energy drinks, soft drinks, cheese/yoghurt (in minutes),
- Percentage of tooth surfaces with recession per subject,
- Percentage of tooth surfaces with bleeding scores per subject,
- BEWE sextant cumulative score per subject.

The subjects occupation (Self-employed, managers, other white collars, manual workers, house person, unemployed, student), whether they suffered from repeated vomiting in the previous 12 months and their number of visits to the dentist in the previous 12 months were only significant for the Schiff sextant cumulative score ($p<0.05$). Brushing after lunch was only significant for the BEWE sextant cumulative

score ($p<0.05$). Subjects who brushed after lunch also brushed after breakfast and dinner.

3.5.10.2 Multiple linear regression analyses

The exposure or predictor factors shown to have statistically significant associations with the Schiff or BEWE sextant cumulative scores in the univariate analysis in section 3.5.10.1 were then used in a multivariate analysis, otherwise known as a multiple linear regression analysis. They were analysed against either the 'Schiff sextant cumulative score' or 'BEWE sextant cumulative scores', which were known as the dependant variables respectively. This model allows analysis of all the exposure factors to be taken into account by simultaneously adjusting for their effects. Coefficients (and their associated confidence intervals and p values) for each exposure factor are shown in sections 7.6 and 7.7. The coefficient indicates how much the outcome for the predictor variable increases or decreases for every 1 unit increase in the dependant variable.

For the dependant variable 'Schiff sextant cumulative score', the statistically significant predictors (coefficient, p value) were;

- BEWE sextant cumulative score per subject (+0.264 $p<0.0001$),
- Percentage of tooth surfaces with gingival recession per subject (+0.049, $p<0.0001$),
- Subject reports current DH is caused by touch stimuli never/rarely/occasionally/often (-0.482, $p=0.017$),
- Subject reports current DH is caused by hot stimuli never/rarely/occasionally/often (-0.901, $p<0.0001$).

For the dependant variable 'BEWE sextant cumulative score', the statistically significant predictors (coefficient, p value) were;

- Subject reports current DH is caused by hot stimuli never/rarely/occasionally/often (+0.684, p=0.048),
- Schiff sextant cumulative score per subject (+0.529, p<0.0001),
- The subject reports brushing after lunch never/rarely/occasionally/often (+0.365, p=0.018),
- Subjects dental practice located in metropolitan, town or rural locations (+0.208, p=0.013),
- Percentage of tooth surfaces with gingival recession per subject (+0.040, p=0.026),
- The subject reports consuming soft drinks often (-0.536, p=0.007),
- Subject reports current DH is caused by touch often/occasionally/rarely/never (-0.574, p=0.034).

There were no statistically significant associations between Schiff sextant cumulative score recorded at the clinical examination and whether the subject considered they currently suffered from sensitive teeth and whether the subject reported experiencing toothache due to sensitive teeth in the previous 12 months (p=0.754).

3.6 Discussion

This study provided data on the prevalence of tooth wear and DH and associated aetiological factors. It used the BEWE sextant cumulative score and Schiff sextant cumulative score, which provide an indication of the severity of tooth wear and DH respectively per subject. The study has shown that there is a positive statistically

significant relationship between the severity of tooth wear recorded using BEWE and the severity of DH recorded using Schiff taken as a sextant cumulative score per subject respectively from a sample of 350 subjects aged between 19 and 34 years old in primary (62.6%) or secondary (37.4%) sites in the South East of England ($p<0.0001$). Their statistically significant positive association show for the first time that as the severity of tooth wear increases, so too does the severity of DH. This would be expected because as tooth wear progresses, the distance from the surface of the tooth to the pulp decreases. The diameter of the dentine tubules is also greater nearest the pulp (Mjor and Nordahl, 1996) as described in section 1.2. Therefore, the hydrodynamic process of DH would be expected to increase (Pashley, 1990a; Pashley, 1994). This helps support the notion of DH as a tooth wear phenomenon. Other smaller studies on 29 patients in Nigeria also showed a statistically significant relationship between DH, tooth wear lesions and gingival recession (Bamise *et al.*, 2008). Agreement between the score for Schiff and BEWE score on all tooth surfaces examined in this study was 79%, but it should be noted that 77.7% (n=18, 711) of tooth surfaces had a BEWE and Schiff score of 0. Furthermore, the proportion of subjects with DH was less than those with tooth wear and this will be discussed.

Reproducibility of tooth wear and DH scoring using the BEWE and Schiff scoring indices respectively on 10% of the randomly selected sample was $\geq$0.96. The population of this study was drawn from a convenience sample of 350. This was to help facilitate recruitment of subjects for this study, who were mostly drawn from general dental practice and secondly from hospital sites in SE England. Subjects were attending for routine dental work. However, the sample was not randomised and is a small sample relative to previous studies mentioned in the literature review. Therefore, this sample may not reflect the prevalence of DH and tooth wear in the wider population. There were no statistically significant differences between male and female,

in agreement with (Deery *et al.*, 2000; Millward *et al.*, 1994), but in disagreement with (Hugoson *et al.*, 1988). Similarly, no statistical differences were observed between socioeconomic backgrounds. Within subject differences in the Schiff and BEWE scores per tooth surface between right and left hand sides of the mouth were frequently reliant on small numbers of subjects where there were differences in the percentages from right to left. Surprisingly a large majority of subjects had the same percentage bilaterally, accounting for very similar medians. This may be expected considering anatomical similarities bilaterally in the periodontal tissues and dietary factors, which affect both sides of the mouth and may have shown greater differences on a larger sample. The sample age group was 18-35. It has been previously mentioned in section 1.4 that DH might be more prevalent in older age groups. Nonetheless, given the small sample size, it was important to restrict age to subjects of a specific age group. Reviews have concluded that DH is most prevalent in age groups 20-40 with a peak towards 30 (Addy, 2000; 2002; Dababneh *et al.*, 1999; West, 2006). In addition, younger age grioups were also more liekly to have less retorative treatment and missing teeth, which would otherwise have been excluded from the analysis. It is of note however that the prevalence of tooth wear would be expected to increase with age and therefore an older age group would nonetheless be of interest for a research study. However, the recent Adult Dental Health Survey (Steele and O'Sullivan, 2009) shows that the prevalence of DH has increased the most in the youngest age groups, between 16 and 44 years old, where wear and in particular moderate wear has recently increased since the previous survey conducted in the UK.

3.6.1 DH

The number of subjects who were examined clinically to have DH on at least one tooth surface was 43.4%, n=152. Other studies, which use a clinical diagnosis, as opposed to subject questionnaire reported DH, have found lower prevalence figures between 4.1% (n=201 subjects) and 2.8% (n=152 subjects) (Rees and Addy, 2002; 2004). However, these previous studies assessed buccal cervical DH only. In this current study, over one quarter of teeth had DH on lingual/palatal and occlusal/incisal surfaces. Furthermore, the previous studies reported on the prevalence of DH in subjects aged 16 to 82 years old (Rees and Addy, 2002) and 15 to 80 years old (Rees and Addy, 2004) respectively, unlike this study which reports on the prevalence in the 18-35 year old age group. It was reported that DH is highest in the 30 to 50 (Rees and Addy, 2002) and 30-40 (Rees and Addy, 2004) year old age groups respectively. Other previous studies also report a peak prevalence in age groups 20 to 25 (Orchardson and Collins, 1987b), 25 to 29 (Graf and Galasse, 1977), 30 to 39 (Rees, 2000; Rees and Addy, 2002) and 31 to 40 (Udoye, 2006). Therefore, the prevalence of DH in the 18 to 35 year old age group would presumably be higher than average. However, some other studies disagree DH is highest in younger age groups, but nonetheless report similar higher prevalence figures for clinically recorded DH. For example in a recent study of 2, 640 subjects in China, the clinical measurement of DH was 25.5% (Que *et al.*, 2010b). Relatively high prevalence figures for DH are also reported in other studies. In a prospective study of similar sample size (n=391), but using randomly selected subjects in Switzerland, 34.8% of subjects reported DH, however the prevalence of DH in those subjects with tooth wear was 84.6% (Lussi and Schaffner, 2000). Therefore, in similarity to the Lussi and Schaffner study, the high prevalence of DH in this study may be in part due to the high-recorded levels of tooth wear (91%).

It should be noted that 59.4% had attended their dentist previously for a check-up, examination or cleaning appointment. It has been mentioned that DH may occur iatrogenically following scaling or root planning (Drisko, 2002), but all subjects were screened prior to their appointment with their dentist. Furthermore, variations in the way the data is presented will affect the prevalence figure. At the tooth surface level, the percentage of tooth surfaces, which were found to have DH, was 3.4%. Nonetheless, most studies report DH at the subject level.

Subject-centred as well as clinical diagnoses were used in this study (Boiko *et al.*, 2010). The subject reported prevalence of DH was higher than the professional clinical diagnosis reported prevalence. Subjects who reported having DH “often” or “occasionally” in the previous 12 months (59.7%, n=209) was greater than subjects who reported having DH currently (44.3%, n=155), which in turn was similar to subjects who were recorded to have DH at their clinical appointment (43.4%, n=152). Therefore, there appears to be a change in the DH recorded over time. Other studies recorded a similar prevalence of DH following subject-based assessment of 41.7% and following clinical measurement was 25.5% (Que *et al.*, 2010b). There were no statistically significant associations between the subject reporting a history of DH, having DH currently or being assessed as having DH clinically using the multivariate analysis ($p=0.754$).

The variation in the presence of DH can be further observed in relation to the aetiological factors. Amongst those subjects who were examined to have DH at their clinical appointment, 87.2% (n=130) had consumed an erosive food or drink within 60 minutes. Of those subjects who did not have DH at their clinical appointment, 84.7% (n=166) had consumed an erosive food or drink more than 60 minutes prior to their appointment. In addition, over 90% of subjects who consumed acidic foods and drinks

more than ten times per day also reported DH. Furthermore, amongst subjects who consumed fresh fruit, fruit and vegetable juice, isotonic and soft drinks two or more times per day, more than 80% reported currently having DH. Over 70% of subjects who delayed brushing less than 20 minutes after breakfast had DH. However in subjects who delayed more than 20 minutes, less than 35% had DH. There were no statistically significant correlations in the multivariate analysis in any of these results. However, it could be inferred that the presence or absence of DH is episodic. As mentioned in section 1.6, the presence of tooth wear does not necessarily lead to the presence of DH, but DH is induced when a tooth wear lesion is “initiated” by a tooth wear process and dentine tubules become un-occluded from the surface of the tooth to the pulp thereby activating the hydrodynamic process (Addy, 2002). The presence or absence of these dietary acidic consumables may be fundamental in initiating DH in these tooth wear lesions and DH could therefore indicate the presence of tooth wear processes. The fact that the DH was reported to last up to 1-5 years in some cases also indicates that the process is ongoing. Recent *in vitro* work recommends delaying brushing for at least 60 minutes after consuming an erosive drink in order to avoid the introduction of surface lesions into dentine (Choi *et al.*, 2012). More work is necessary on the episodic nature of DH and to relate this to the presence of aetiological factors in tooth wear clinically and in the laboratory.

The fact that DH was less likely on lingual/ palatal (26%) and occlusal/ incisal (28%) tooth surfaces than on buccal (43%) tooth surfaces is indicative that DH is more prevalent on specific tooth surfaces, perhaps because erosion as opposed to other forms of tooth wear, is most active. On lingual/palatal surfaces, the combination of abrasion from the tongue (as well as abrasion from tooth brushing) may act to create a smear layer blocking the dentine tubules. Equally, in the occlusal surfaces, there is also a combination of attrition from tooth to tooth contacts (Bartlett, 2005b). This may also

help explain why there was more tooth wear recorded occlusal surfaces (81%) compared to buccal (73%) and lingual/palatal (25%) surfaces. All tooth surfaces were examined in this study because DH is often linked to tooth wear and this can occur on any surface (Bartlett, 2005b). One study investigating all tooth surfaces in 2,165 patients in Nigeria observed more DH in occlusal surfaces (Bamise *et al.*, 2007). The prevalence of DH was only 1.34% and hence low compared to previous large studies on DH, however the higher frequency of DH on occlusal surfaces suggests reflects an importance to include these surfaces in prevalence studies. On occlusal surfaces, attrition as well as abrasion may contribute more DH if it is in combination with erosion. Furthermore, a later study on 29 patients showed that gingival recession, followed by attrition, were the most important aetiologies in DH (Bamise *et al.*, 2008). However these studies did not use patient questionnaires to investigate dietary habits in this Nigerian population and relied on clinical diagnosis of wear lesions alone. In similarity to our study, gingival recession was statistically significantly associated with the Schiff sextant cumulative score ($p<0.001$). Like tooth wear, gingival recession exposes dentine and is associated with DH (Addy *et al.*, 1987d) due to its involvement in "localising" a dentine wear lesion which may then lead to a hydrodynamic process (Addy, 2002).

Subjects who had more severe DH recorded at their clinical appointment using the sextant score were less likely to have reported sensitivity due to touch ($p=0.017$) or hot stimuli ($p<0.0001$). The stimulus used for the Schiff and DH indices in this experiment was evaporative therefore DH may have been over represented in this instance. Nonetheless, tactile sensitivity assessed as a probe applied to the cervical region of teeth was not assessed as previous research has shown that there is no difference in the subjective response to tactile and evaporative stimuli (Chabanski *et al.*, 1997). In addition, although 51.2% and 32.9% of subjects reported current DH due to touch and

hot stimuli respectively, a much larger proportion had DH due to other stimuli, including tooth brushing (78.2%), cold drinks (64.1%) and cold weather (62.8%). The evaporative stimulus is the most frequently used stimulus for evaluating DH (Klineberg *et al.*, 1990) and considered the most similar to DH induced naturally. The evaporative stimulus is dominant but there is also thought to be a combination from thermal stimuli, the latter depending on duration and temperature although the stimulus was limited to 1 second blasts as recommended in the literature (Pashley, 1990c). It has also been recommended that evaporative or thermal stimuli be used in the guidelines for clinical trials on DH (Holland *et al.*, 1997; West *et al.*, 1997). However, Holland recommended that two different hydrodynamic stimuli be used and that there be a reasonable time gap between stimuli with the less severe stimuli applied first (Holland *et al.*, 1997) because DH has been shown to change for different stimuli (Orchardson and Collins, 1987a). Despite this, such a time gap has not been defined and limited data is available to establish the reproducibility of these stimuli (Ide *et al.*, 2001). The stimuli used in our study were quantifiable, using a semi-subjective judgement of the subject's pain related behaviour recorded by the examiner. They were also clinically relevant and were shown to be reproducible. These characteristics have all been described as important for stimuli used in the measurement of pain (McGrath, 1986). However, more recent work has shown that the expectation of a response to the stimulus might prompt a heightened pain response and has suggested that more research needs to be undertaken on the accuracy and reliability of the test stimuli used to elicit DH in clinical studies and trials (Addy *et al.*, 2007).

DH was found more commonly on first molars, followed by second premolars, then first premolars, first incisors, canines and finally second incisors. To some extent, other studies support that molars, followed by premolars/canines and finally incisors are the

most commonly affected teeth (Chabanski and Gillam, 1997; Rees *et al.*, 2003; Rees and Addy, 2004).

Subjects who had DH often or occasionally in the previous 12 months were more likely to have experienced various life events, which are indicative of anxiety or depression ($p<0.05$). There were no statistically significant associations with the lifestyle events and the Schiff sextant cumulative score using in the multivariate analysis. Of those who reported current DH, 57% mentioned his was important or very important. Previous research has shown that DH impacts on oral health related quality of life (Bekes *et al.*, 2009) and everyday life including oral hygiene (Gibson *et al.*, 2010).

3.6.2 Tooth wear

Most subjects had tooth wear on at least one tooth surface (91%, n=319), with 47.2% having a distinct defect and 10% (n=35) of subjects having advanced tooth wear that affected more than 50% of at least one tooth surface. These values are different to the proportion of subjects who had DH and are indicative of the observation that a tooth wear lesion will not necessarily indicate DH unless the dentine tubules are also exposed by tooth wear processes (Addy, 2002). The results of tooth wear are similar to previous studies, which have found 98% tooth wear on 93, 500 tooth surfaces in 1, 007 adults and levels of advanced wear were 5.73% using the Smith and Knight Tooth Wear Index (TWI) (Smith and Robb, 1996). It has been reported in the literature that pathological tooth wear could be underestimated using the TWI, because the threshold levels for each age group for which tooth wear were compared to were high (Bardsley, 2008). A systematic review of the literature reports that the prevalence of severe tooth wear can affect up to 17% of subjects (Van't Spijker *et al.*, 2009), but it should be noted that this affected mostly older age groups and that at age 20 the prevalence was closer

to 3%. In addition, in our study location was a significant predictor for the BEWE sextant cumulative score (p=0.013). Tooth wear was higher in second compared to first molar teeth, however in second molars this was predominately a BEWE score 1 whereas first molars had a higher BEWE score 2, on average. The BEWE 1 score reflects early wear which is not a distinct defect and often occurring in enamel.

Subjects who reported that they currently had DH caused by touch (51.2%, n=80) were less likely to have wear recorded using the BEWE sextant cumulative score in the multivariate analysis. Touch is a mechanical process and would be expected to cause DH if the dentine tubules are exposed and patent to the pulp. Erosion is strongly implicated in removing the smear layer and exposing un-occluded dentine tubules. In our study, 64% (n=224) of subjects reported consuming acidic food and drinks often or occasionally (Bartlett, 2005b). Subjects with more DH caused by touch may therefore avoid touching and abrading those areas of the tooth which are more sensitive, but not if the dentine tubule system is occluded perhaps due to the presence of secondary dentine or reduction in dietary erosive beverages for example. In contrast, subjects who reported current DH caused by hot stimuli were more likely to have tooth wear (p=0.048). This is marginally significant (p<0.05) and may be due to the reduced thermal transfer in wear lesions where the distance to the pulp is less (Linsuwanont *et al.*, 2008).

Subjects who reported consuming soft drinks often were more likely to have a lower BEWE sextant cumulative score (p=0.026) in the multivariate analysis. This finding may in part be explained by how the drinks are consumed rather than how often and perhaps an individual subject's awareness of the acidity of these drinks. The same result was not found for the other acidic drinks, such as fruit and vegetable juice and fresh fruit. The higher acidity of fruit juices in comparison with soft drinks such as the

Coca-Cola drink has been discussed in section 1.7.2. In addition, for those subjects who reported consuming soft drinks often, 53% consumed these twice a day or more, in contrast to ≥68% of subjects who reported consuming fresh fruit, isotonic drinks or fruit and vegetable juice often. However, there was not a statistical significant relationship with consuming fresh fruit and fruit or vegetable juice more often with increasing levels of tooth wear in this study. Again, this may be related to method of consumption rather than frequency. In addition, most of the subjects used fluoride toothpaste (n=323, 92%). Although fluoride was not significant in the multivariate analysis for either BEWE or Schiff sextant cumulative, it has been shown to harden the tooth surface and increase its resistance to acid dissolution (Bartlett, 2005b).

Brushing after lunch was linked to more tooth wear (p=0.018) and these subjects also brushed after breakfast and dinner. Indeed, increasing brushing frequency or overzealous brushing, has been shown to lead to increased levels of tooth wear in addition to causing gingival recession and, in turn, could lead to more DH (Addy and Hunter, 2003; Addy, 2005). The percentage of buccal and palatal tooth surfaces with gingival recession was a significant predictor for the BEWE sextant cumulative score (p<0.026), as well as Schiff sextant cumulative score (p<0.001). In addition, manual tooth brushes have shown to cause more wear than electric tooth brushes (Van der Weijden *et al.*, 2011) and in this study almost two thirds of subjects (62.3%, n=232) used manual toothbrushes.

3.6.3 Conclusion

In conclusion, this study refutes the null hypothesis that there is no association between tooth wear and DH. As the severity of tooth wear increases, so too does the severity of DH recorded clinically (p<0.0001). There was also a positive relationship

between gingival recession, tooth wear ($p=0.026$) and DH ($p<0.0001$). There were not statistically significant associations between the Schiff sextant cumulative score and BEWE sextant cumulative score and all the various aetiologies using the multivariate analysis, in agreement with the null hypothesis. However, more DH was observed in this small sample of subjects (n=350) with increasing frequency or recent consumption of acidic food or drink.

Chapter 4 An *in situ* study investigating dentine tubule occlusion of dentifrices following acid challenge

4.1 Aim

The aim of this study was to investigate the dentine occlusion and acid resistance of two dentifrices developed to treat DH using an established visual ordinal scale for measurement of tubule occlusion.

4.2 Null hypothesis

The null hypotheses were that both dentifrices did not occlude the dentine tubules and were soluble in acid.

4.3 Method

4.3.1 Dentine samples

Caries free human third molars recently extracted, intact, from patients aged over eighteen years, of either gender were used to produce 448 samples for the study as described in section 2.2. This was in accordance with ethical approval Z4010980 by the South West London Research Ethics Committee. TSM was used to confirm sample integrity and establish tubule density and orientation visually prior to placement *in situ*. This helped ensure all samples were prepared with an even distribution of patent, cross-sectioned tubules. In total, 718 dentine samples were made, but 270 were excluded as the dentine tubules were not at the correct orientation or un-occluded when examined using TSM. Dentine samples were identified with a unique number.

4.3.2 *In situ* model

The clinical study employed a single centre, single blind (blinded to the person responsible for performing the dentine sample analysis), randomised, four-treatment split mouth, two period and crossover design *in situ*. Consent forms and patient information sheets are available on request. Healthy subjects aged eighteen or above, able to accommodate bi-lateral appliances and compliant with study procedures and restrictions were screened within 35 days prior to the start of the first treatment period. Oral soft tissue examinations and adverse event reporting were performed at screening and follow up appointments (seven days post treatment) to ensure subjects' tolerance of study procedures. Typical exclusion criteria included susceptibility to acid regurgitation, caries or periodontal disease or any condition or medication causing xerostomia. Standard toothpaste (Crest Decay Prevention, Proctor and Gamble, Weybridge, UK) and brush (Oral B Indicator 35, Soft Toothbrush, Gillette, Proctor and Gamble, Weybridge, UK) were used by subjects from screening to follow up visits and a washout period of 48 hours occurred between treatment periods to reduce cross over effects.

Left and right acrylic appliances, secured to the buccal surfaces of lower molars and premolars, were designed to securely hold four dentine samples whilst allowing access for sample removal and placement (as described in section 2.5.2). Subjects wore the intra-oral appliances over two treatment periods, each of four consecutive weekdays (Monday to Thursday inclusive) for a minimum of five hours daily. Whilst appliances were *in situ*, no food or drink was consumed and subjects abstained from smoking and chewing gum. Appliances were removed for up to one hour at lunchtime and stored in a moist container. During each study period 1.10 grams (g) ± 0.28 g of toothpaste or

50ml of water were brushed onto each appliance with an Oral B® Vitality Precision Clean Power Toothbrush fitted with EB 17 (flexisoft) Brush Head Refills (Oral B EB17 Flexisoft, Weybridge, UK) (treatment and subject specific to prevent cross contamination) *ex vivo* for ten seconds twice daily at 10:00 h ± 30 and 14:30 h ± 30 min by a single examiner. Treatments included;

- Sensodyne Rapid Relief® (GlaxoSmithKline Consumer Healthcare, Weybridge, UK) containing (8% Strontium acetate, 0.24% Sodium Fluoride). White paste.
- Colgate Sensitive Pro-Relief® (Colgate-Palmolive (UK) Limited, Guildford, UK) containing Pro-Argin™ formulae (with 8% Arginine and Calcium Carbonate as well as 1450ppm or 1.1% Sodium monofluorophosphate). White paste.
- Control Toothpaste (GlaxoSmithKline Consumer Healthcare (GSKCH), Weybridge, UK) containing 0.32% Sodium Fluoride. White paste.
- Volvic® Still Mineral Water Groupe Danone SA, Paris, France.

Dentifrices were overwrapped to maintain the study blind. The temperatures of storage and treatment rooms were monitored throughout the study and were 21°C ± 3°C.

Appliances were worn at least sixty minutes prior to and following the first and last treatments each day. Over both treatment periods, all four treatments were tested on each subject.

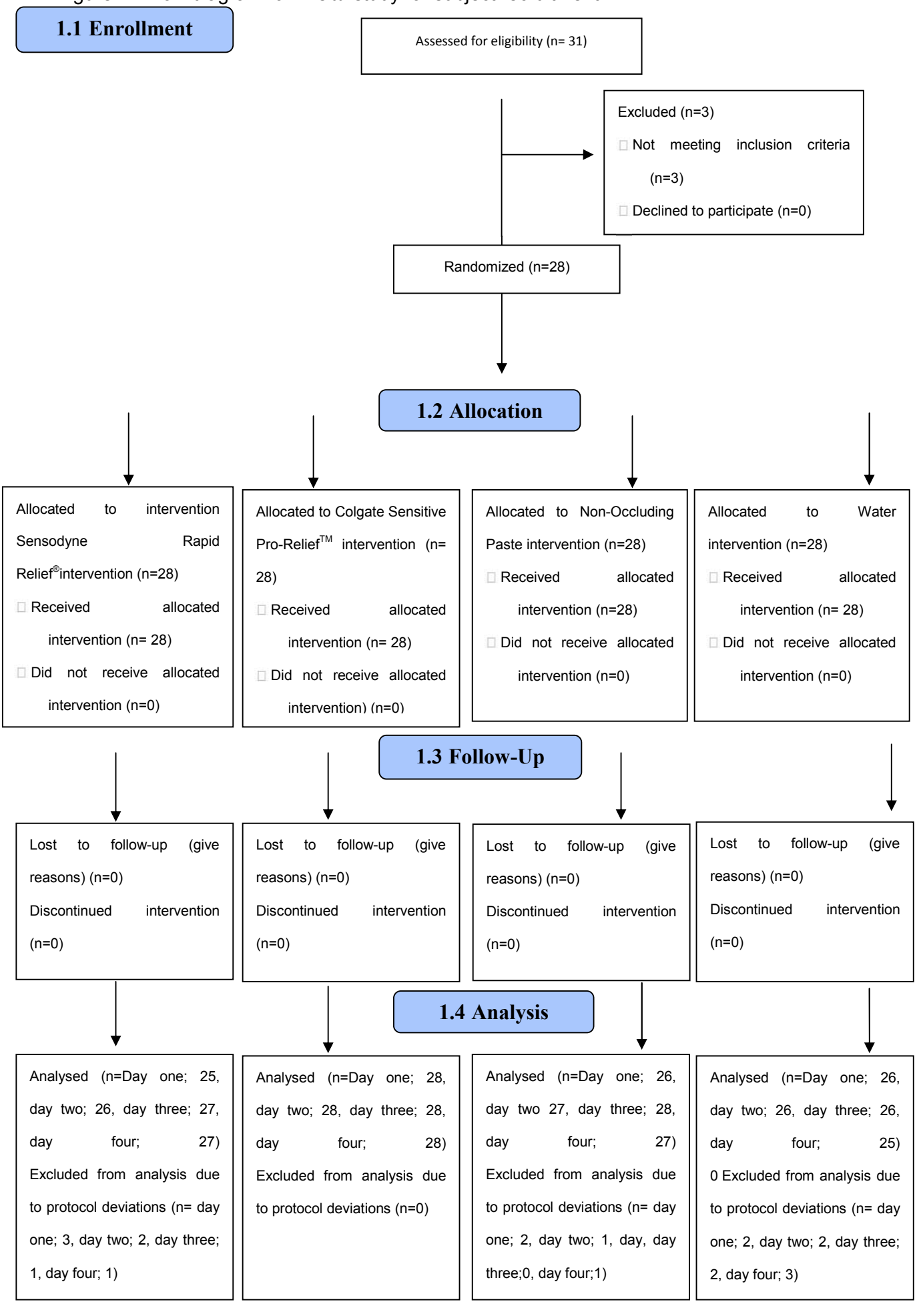
An acid challenge was applied *ex vivo* on days three and four of each treatment period. This occurred twice a day commencing 60 minutes following treatment with toothpaste. Appliances were each placed in Tesco Pure grapefruit juice (Smooth) for one minute under gentle agitation using a mini orbital shaker S05 (Stuart Scientific, UK) and then rinsed in Volvic® bottled water.

4.3.3 Evaluation of samples

At the end of each treatment day, one sample was removed from each appliance and stored dry for subsequent SEM analysis. At the end of the study all the samples were fixed to SEM pin stubs and gold sputter coated for SEM imaging. The same member of staff who was blinded and independent from grading took SEM images from the centre of each dentine sample. Previously trained, calibrated and blinded judges then scored the SEM images of the teeth using the visual ordinal scale for SEM images (described in section 2.3). A mean score was then calculated per sample.

The flow diagram of subject recruitment during the *in situ* study is shown in Figure 42.

Figure 42 Flow diagram for *in situ* study for subject recruitment



4.3.4 Statistical analysis

Assuming a standard deviation of the difference of 0.843 units (established from published studies (Claydon *et al.*, 2009; Parkinson and Willson, 2011a)) and the use of a two sided 5% significance test, 25 evaluable subjects were required to detect a difference of at least 0.5 units between treatments with 80% power. Allowing for dropouts and protocol violations, a sufficient number of subjects were screened in order to randomise 28 subjects to ensure 25 subjects were evaluable. Treatment period duration and evaluation time points were again based on previous studies (Claydon *et al.*, 2009; Parkinson and Willson, 2011a).

The mean scores each day for each treatment were then compared using analysis of variance (ANOVA) based on a mixed model with factors of subject, treatment, period and side of mouth. Subject was included as a random factor. All treatment comparisons were performed at the 5% significance level using two-sided testing with no adjustment for multiplicity. The influence of acid challenge was assessed based on the change in occlusion score between day two and day four for each treatment.

4.3.5 Cross sectional analysis

Energy dispersive X Ray Crystallography (EDX) was used to confirm the constituents of the dentifrices used in this study. Table 28 shows EDX data taken from each dentifrice under investigation. The percentage by weight of elements in the 8% arginine dentifrice was similar to the control paste. In the 8% strontium dentifrice, the proportion of silica by weight was greater (7.36%) compared to 8% arginine and control paste (1.00%) and similar to the percentage by weight of strontium (7.99%).

Table 28 EDX data taken from 8% strontium based dentifrice (left) b) 8% arginine based dentifrice (middle) and control paste (right). C=Carbon, O=Oxygen, Na=Sodium, Si=Silica, S=sulphur, Sr=Strontium.

8% strontium dentifrice		8% arginine dentifrice		Control paste	
Element	Weight%	Element	Weight%	Element	Weight%
C	29.90	C	45.91	C	45.91
O	61.72	O	44.96	O	44.96
Na	1.02	Na	4.34	Na	4.34
Si	7.36	Si	1.00	Si	1.00
Sr	7.99	S	3.79	S	3.79

Pilot work took place following the *in situ* study, in order to evaluate the constituents of the deposits formed within the dentine samples and their depth of penetration into the tubules. Initially, nine dentine samples were randomly selected from those used in the *in situ* study and were dry fractured. Samples were fractured, using a scalpel held at 90 degrees to the surface, to reveal two cross-sectioned surfaces. The scalpel fractured each specimen from the underneath (thus avoiding the test surface). A diamond wafer blade was not used to avoid creation of a smear layer. The cross sections were examined using TSM in conjunction with an M-Plan 20x SLWD Bright field Objective x20/0.35 NA water based objective). These investigations did not reveal differences between the treated dentine samples. A further nine fractured samples were then randomly selected from those used in the *in situ* study, from different treatment groups and days. One half of each fractured sample was then coated with carbon for EDX analysis and imaging. The remaining half of the same sample was gold coated for subsequent SEM imaging.

4.4 Results

4.4.1 Demographics

In total, 31 subjects were screened, of whom 28 were randomised with all 28 completing the study. This included twelve (42.9%) males and sixteen (57.1%) females with a mean age of 34.7 years (SD 8.41 years). In addition, nineteen (67.9%) were white, six (21.4%) were Asian and three (10.7%) were of black or African origin. All 28 subjects were evaluable. Review prior to un-blinding identified eight protocol deviations with the potential to affect efficacy recorded across six subjects, which each led to exclusion of their specific data only on specific treatment days. For example, consuming diet Pepsi at lunch (while the appliance was not *in situ*). The data for the mean visual ordinal grade showed a normal distribution.

4.4.2 Visual ordinal scale results

Figure 43 presents adjusted mean scores for each treatment and associated between-subject standard errors for the primary and secondary analyses. Table 29 presents between-treatment differences with associated confidence intervals and p-values for the same endpoints. Figure 44 shows SEM images of dentine samples each day selected from the average scores.

Figure 43 shows that 8% strontium acetate had a lower adjusted mean score on day one (1.91 (SE 0.196)), day two (1.92 (SE 0.179)), day three (1.82 (SE 0.176)) and day four (1.65 (SE 0.176)) compared to all other products except 8% arginine (2.08 (SE 0.172)) on day two.

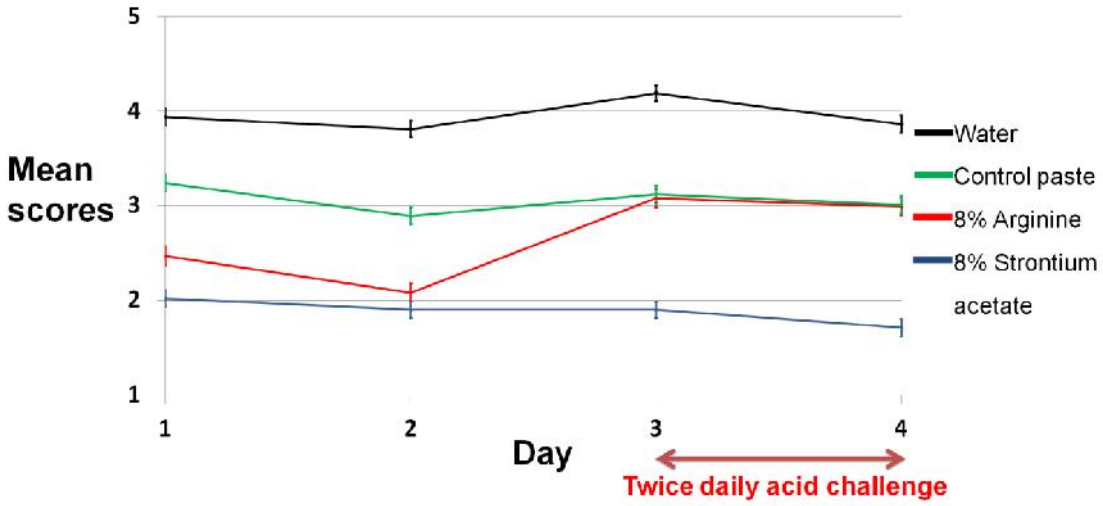


Figure 43 Adjusted means and associated between-subject standard errors from ANOVA model including subject as a random effect, and treatment, period and location of sample in mouth (left or right) as fixed effects. The mean score is of the visual ordinal scale: 1=occluded, 2=partially un-occluded, 3=equally occluded/un-occluded, 4=partially occluded, 5=un-occluded.

Table 29 shows that the adjusted mean scores for 8% strontium acetate were statistically significantly lower compared to all other treatments ($p<0.05$), with the exception of 8% arginine on day two where no significant difference was observed ($p=0.5143$). This is reflected in Figure 44, which demonstrates a low number of visible un-occluded dentine tubules from day one to four for the 8% strontium acetate compared to the control paste and water. Although surface coverings were not typically seen on 8% strontium acetate treated samples, there are fewer numbers and diameter of dentine tubules visible.

Table 29 Differences in mean visual ordinal grade and associated 95% confidence intervals and p-values from ANOVA model including subject as a random effect, and treatment, period and location of sample in mouth (left or right) as fixed effects. Differences are first named treatment minus second named treatment such that a negative difference favours the first named treatment.

* Twice daily 1min acid challenge introduced.

Day	Comparison of Treatments	Diff.	95% Confidence Interval	P-Value
1	8% Strontium Acetate Toothpaste vs. Water	-2.00	(-2.54, -1.46)	<0.0001
	8% Strontium Acetate Toothpaste vs. Control Paste	-1.39	(-1.93, -0.85)	<0.0001
	8% Strontium Acetate Toothpaste vs. 8% Arginine Toothpaste	-0.56	(-1.09, -0.03)	0.0372
	Control Paste vs. 8% Arginine Toothpaste	0.83	(0.31, 1.35)	0.0023
	Water vs. 8% Arginine Toothpaste	1.44	(0.91, 1.96)	<0.0001
	Control Paste vs. Water	-0.61	(-1.14, -0.08)	0.0259
2	8% Strontium Acetate Toothpaste vs. Water	-1.87	(-2.37, -1.37)	<0.0001
	8% Strontium Acetate Toothpaste vs. Control Paste	-0.95	(-1.44, -0.45)	0.0003
	8% Strontium Acetate Toothpaste vs. 8% Arginine Toothpaste	-0.16	(-0.65, 0.33)	0.5143
	Control Paste vs. 8% Arginine Toothpaste	0.78	(0.30, 1.27)	0.0019
	Water vs. 8% Arginine Toothpaste Daily Paste	1.71	(1.22, 2.20)	<0.0001
	Control Paste vs. Water	-0.93	(-1.42, -0.43)	0.0003
3*	8% Strontium Acetate Toothpaste vs. Water	-2.36	(-2.85, -1.86)	<0.0001
	8% Strontium Acetate Toothpaste vs. Control Paste	-1.30	(-1.79, -0.81)	<0.0001
	8% Strontium Acetate Toothpaste vs. 8% Arginine Toothpaste	-1.27	(-1.75, -0.78)	<0.0001
	Control Paste vs. 8% Arginine Toothpaste	0.04	(-0.45, 0.52)	0.8841
	Water vs. 8% Arginine Toothpaste Daily Paste	1.09	(0.60, 1.58)	<0.0001
	Control Paste vs. Water	-1.05	(-1.55, -0.56)	<0.0001

Day	Comparison of Treatments	Diff.	95% Confidence Interval	P-Value
4*	8% Strontium Acetate Toothpaste vs. Water	-2.27	(-2.76, -1.77)	<0.0001
	8% Strontium Acetate Toothpaste vs. Control Paste	-1.41	(-1.89, -0.93)	<0.0001
	8% Strontium Acetate Toothpaste vs. 8% Arginine Toothpaste	-1.35	(-1.83, -0.87)	<0.0001
	Control Paste vs. 8% Arginine Toothpaste	0.06	(-0.41, 0.54)	0.7904
	Water vs. 8% Arginine Toothpaste	0.92	(0.43, 1.41)	0.0003
	Control Paste vs. Water	-0.86	(-1.35, -0.36)	0.0009

Nb. Diff. (difference)

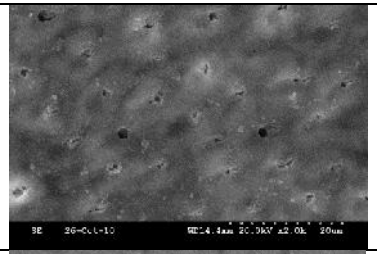
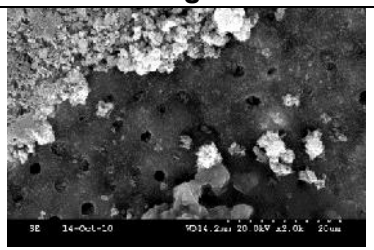
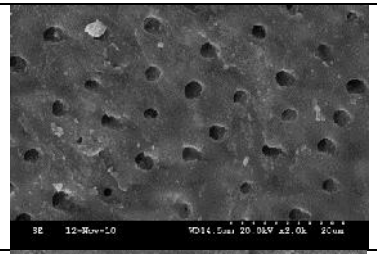
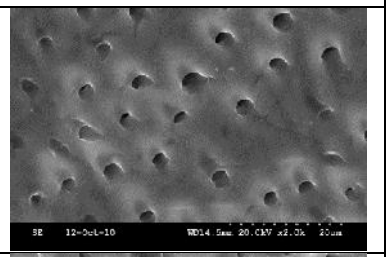
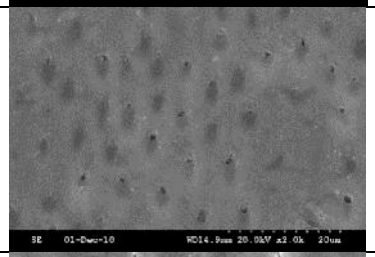
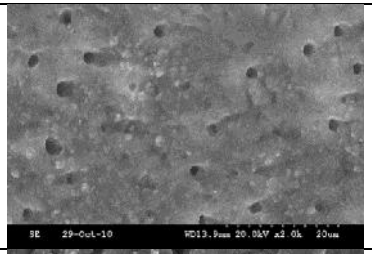
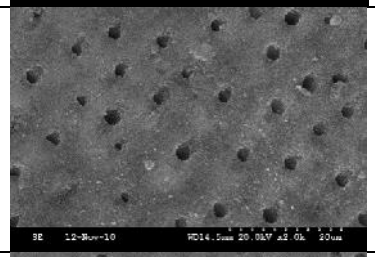
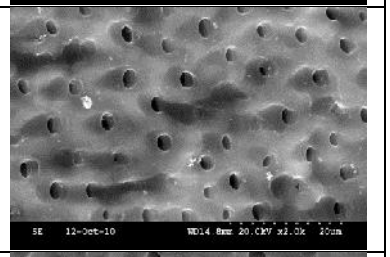
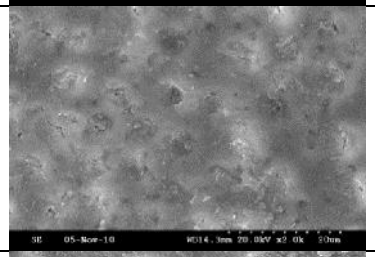
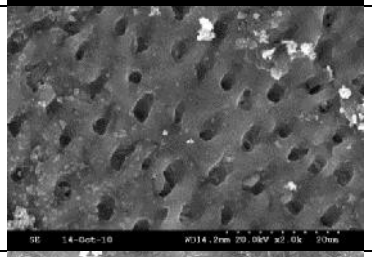
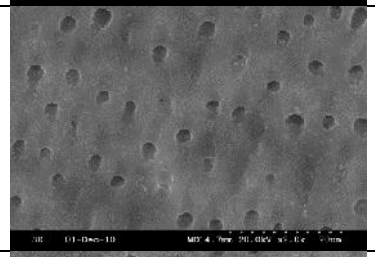
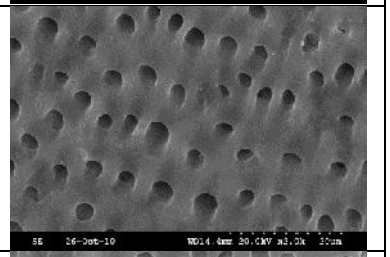
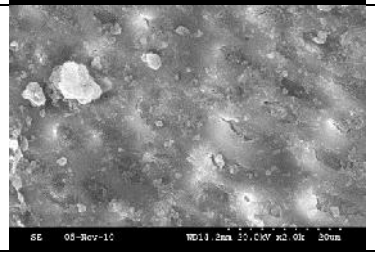
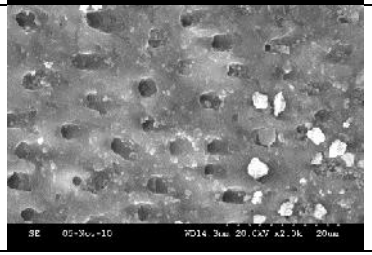
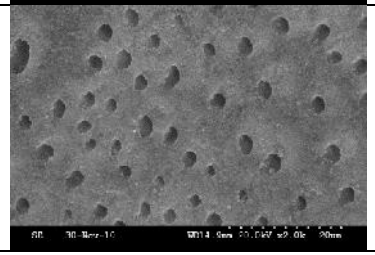
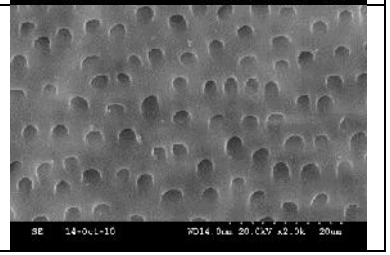
Day	Treatment			
	8% Strontium Acetate	8% Arginine	Control toothpaste	Water
1				
2				
3				
4				

Figure 44 Representative SEM images of dentine samples during each treatment day and product (x 2, 000)

The 8% arginine showed a higher mean score on day one (2.47 (SE 0.185)) than 8% strontium acetate with visibly more patent tubules (Figure 43 and Figure 44 respectively). The mean scores for the control paste and water remained greater than 8% strontium acetate on all days and greater than 8% arginine with visibly more patent tubules on days one and two (Figure 43 and Figure 44).

The mean score for 8% arginine treatments increased from 2.08 (SE 0.172) on day two to 3.08 (SE 0.173) on day three and then decreased slightly to 2.99 (SE 0.173) on day four. Table 29 shows that on days three and four, the 8% strontium acetate had statistically significantly lower scores compared to all products ($p<0.0001$). The 8% arginine had a statistically significantly lower mean scores compared to the control paste (on days one and two) and water (on all days) ($p<0.05$). No statistically significant differences were detected between the 8% arginine and the control paste following acid challenge on days three and four ($p=0.8841$, $p=0.7904$ respectively). Control paste had significantly lower mean scores compared to water on all days ($p<0.05$).

To assess the impact of acid challenge, changes in scores from day 2 to day 4 were calculated ((8% strontium (-0.24 (SE 0.206)), 8% arginine (0.91 (SE 0.202)), control (0.16 (SE 0.206)), water (0.08 (SE 0.214))). Negative changes indicated an increase in tubule occlusion and positive changes indicated a reduction in tubule occlusion. Only the change for the 8% arginine treatment (reduction in tubule occlusion) was statistically significant ($p<0.0001$).

4.4.3 Cross sectional analysis

Figure 45 show SEM images x4000 of the lateral sections of some of the above fractured samples. The corresponding SEM images taken of the treated dentine surfaces prior to fracture of the dentine sample are shown in Figure 46. The cross sections in Figure 45 reveal the appearance of tubular occlusion close to the surface of the dentine, or a surface layer covering dentine, which is more common in those samples having fewer patent tubules (Figure 46). The samples treated with 8% strontium reveal a surface covering on days two (pre-acid challenge), three and four (post acid challenge). Samples treated with 8% arginine based dentifrice reveal patent tubules on days one and two (pre-acid), but not day four (post acid). Samples treated with control paste reveal patent tubules near the surface of the dentine sample on days two (pre-acid) and three (post acid). Dentine samples treated with water reveal patent tubules on days one (pre acid) and four (post acid).

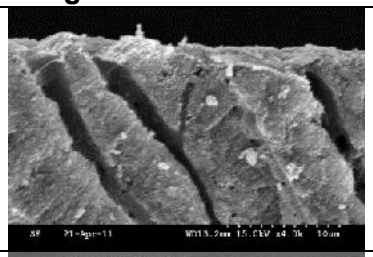
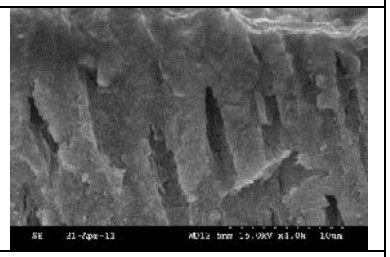
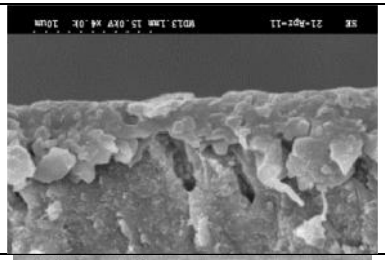
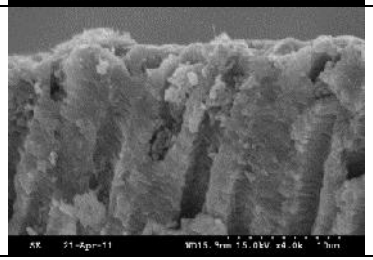
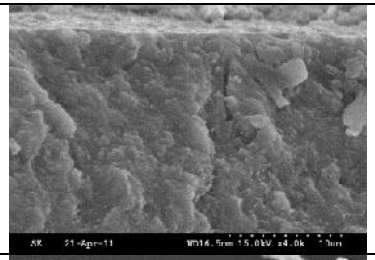
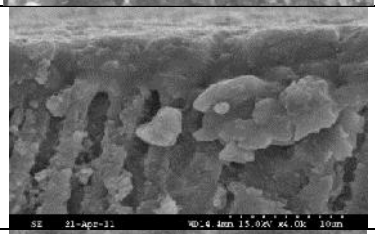
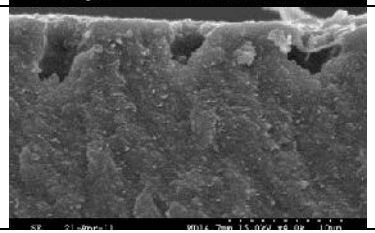
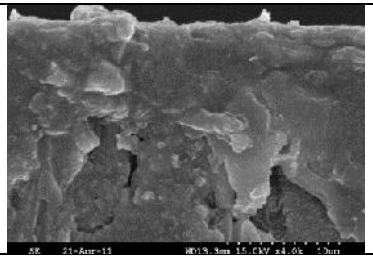
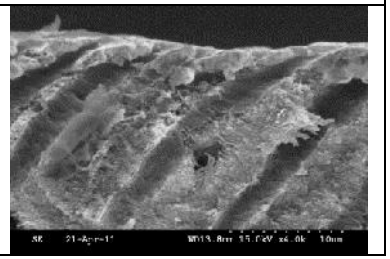
Day	Treatment			
	8% strontium based	8% arginine based dentifrice	Control Paste	Water
1				
2				
3				
4				

Figure 45 SEM images of lateral sections of fractured dentine samples for various treatment days and product (x4000). The surface of each sample is uppermost on each image.

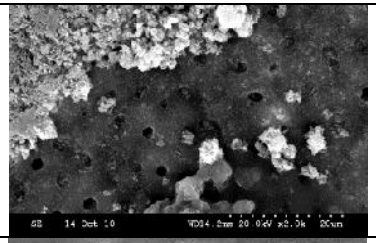
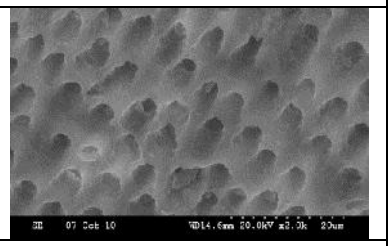
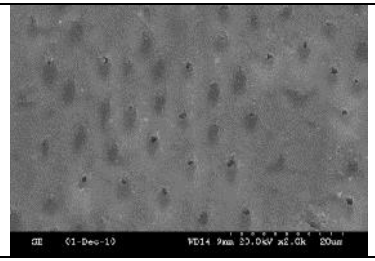
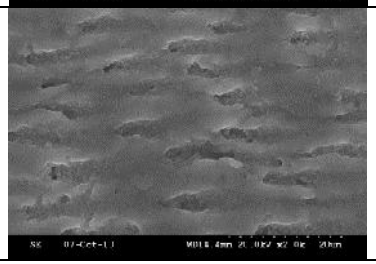
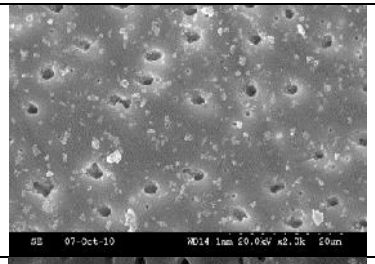
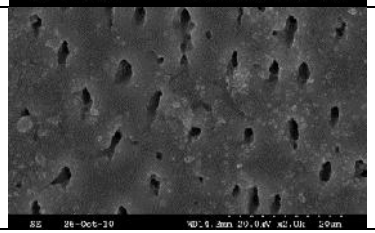
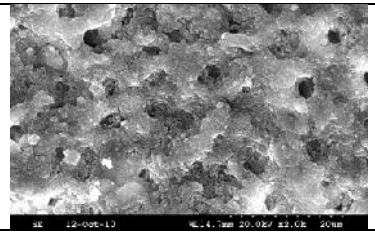
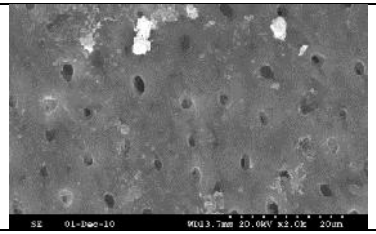
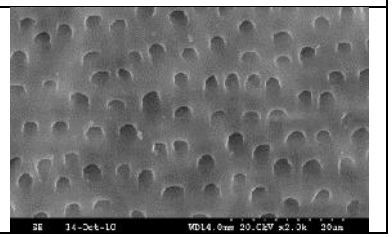
Day	Treatment			
	8% strontium based	8% arginine based	Non-Occluding Paste	Water
1				
2				
3				
4				

Figure 46 SEM images of surface of treated dentine samples various treatment days and product (x2000)

Figure 47 shows an area of cross-sectioned treated dentine samples close to the surface of the dentine samples treated with:

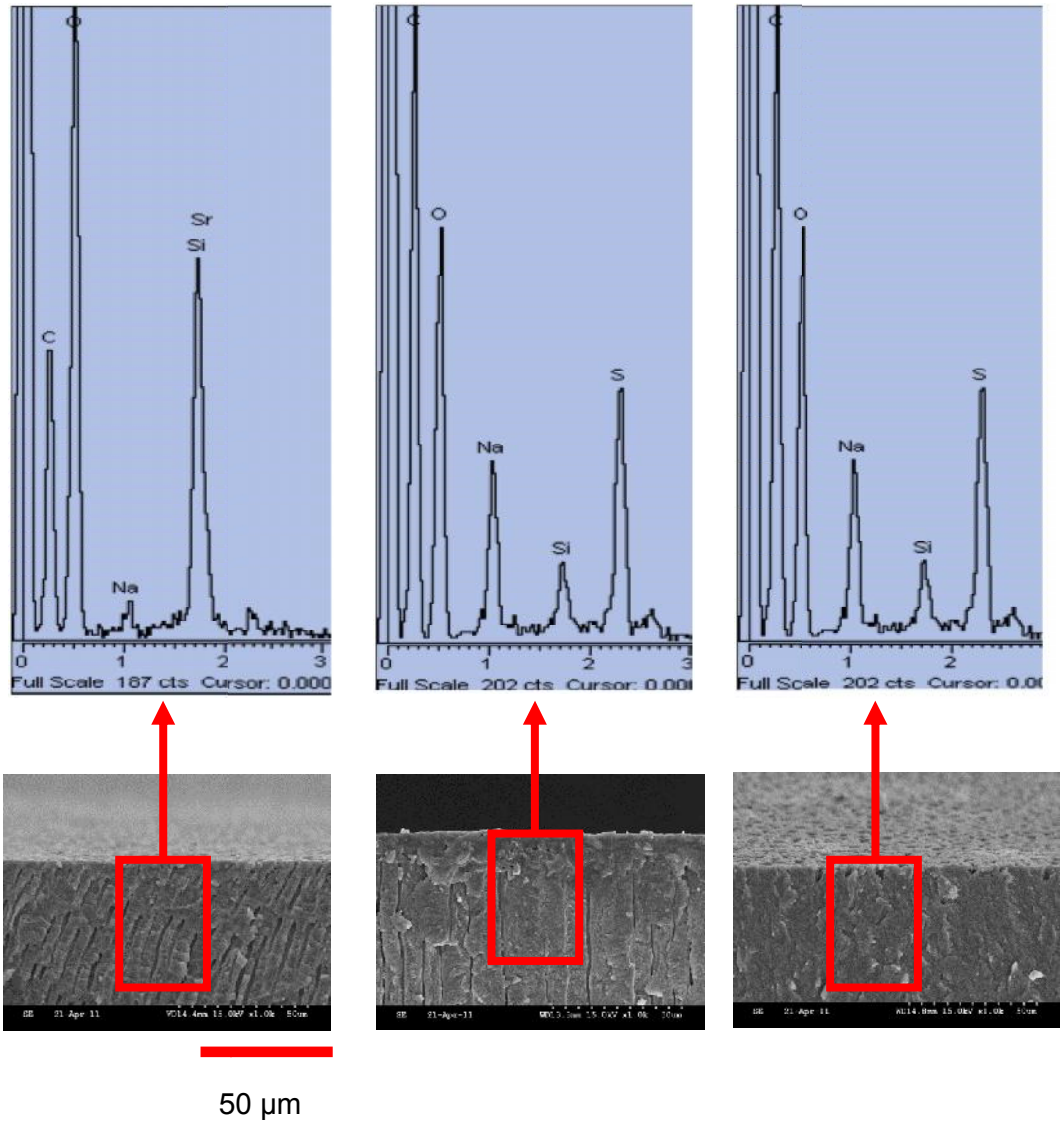
- 8% strontium based dentifrice treated sample at day three,
- 8% arginine based dentifrice treated sample at day four,
- Control paste treated sample at day three.

In the 8% strontium treated sample at day three, the proportion of strontium is highest nearest the surface of the treated sample (1.56%). At 50μm from the surface of the dentine sample, strontium is detected from 1.03% to 0.77%. EDX data from the 8% strontium treated dentine samples at days two and four revealed 0.12% and 0.18-0.17% of strontium respectively at various depths throughout the sample cross sections. The percentages on day three, but not days two and four were far higher in comparison with the dentine samples treated with other treatments, which contained up to 0.15% strontium.

Samples treated with 8% arginine revealed the presence of calcium, phosphate and carbonate, but no measurable differences were observed between calcium, phosphate and carbonate between 8% strontium and 8% arginine treated samples. The proportion of silica in the 8% strontium treated sample was higher than in the 8% arginine treated sample.

EDX data for the non-occluding control paste reveal no clear differences between the calcium and phosphate compared to those dentine samples treated with 8% strontium and 8% arginine.

Figure 47 Elemental analysis (top) using EDX of an area of cross sectioned dentine sample (bottom) treated with 8% strontium at day three (left), 8% arginine at day four (middle), control paste at day four (right). Si=Silica, Sr=strontium, Na=Sodium, C=Carbon, O=Oxygen, S=Sulphur, Si=Silica.



4.4.4 Safety results

Five subjects each reported five treatment-emergent Adverse Events (AEs), three of which were oral events ('oral herpes'). All five treatment-emergent AEs were considered mild in intensity, not related to study treatment and had resolved by the end of the study.

4.5 Discussion

This study was designed to investigate the acid resistant property of dentifrices designed to treat DH, using an *in situ* model and visual ordinal scale to grade dentine tubule occlusion. It has shown that after two days of treatment in an *in situ* model (twice daily brushing), both dentifrices (8% strontium acetate and 8% arginine) demonstrated significantly greater levels of dentine occlusion than both the control dentifrice and water. After the agitated dietary acid challenge (on days three and four), the 8% strontium acetate also demonstrated significantly greater levels of dentine occlusion than all the other products tested. The 8% strontium acetate based dentifrice appears to occlude the dentine with an acid resistant layer.

On days one and two, the 8% strontium acetate and 8% arginine dentifrices demonstrated significantly better dentine tubule occlusion than both water and control paste as shown by a lower mean score in the SEM grading for these dentifrices. This is expected because the active ingredients found in both dentifrices have strong absorptive capacities to dentine. Clinically, arginine works by absorbing onto the surface of calcium carbonate forming positive charged alkaline agglomerate (Petrou *et al.*, 2009). This alkaline agglomerate has a high affinity to dentine and relies on the deposition of calcium and phosphate from saliva to occlude the dentine tubules (Petrou

et al., 2009). The presence of saliva is therefore essential in the mechanism of action of arginine. This is in contrast to the mechanism of action of strontium-based dentifrices. Strontium is an alkaline earth metal from the periodic table, which has strong inherent absorptive capacity for calcified tissues and especially those with a high organic content such as dentine (Hodge *et al.*, 1946). This may be due to its high permeability and possibility for adsorption into or onto organic connective tissues including odontoblasts processes as shown in an early study using the metallic compound strontium chloride (Ross, 1961).

Our finding that both 8% strontium acetate and 8% arginine-based dentifrices result in significant dentine occlusion compared with negative controls is supported by a previous study conducted *in vitro* using similar materials and methodology (Parkinson *et al.*, 2010). This laboratory study investigated the level of dentine tubule occlusion afforded by these dentifrices on bovine dentine discs following twice daily brushing applications using human saliva. Samples were imaged by SEM and scored using the same ordinal scale, which is used in our study. On day two, the difference in the mean score between the 8% strontium acetate and 8% arginine was statistically significant in the laboratory study ($p<0.0021$) compared to this clinical study ($p<0.5143$). During laboratory studies, the application, treatment and other conditions can be far more controlled, and exaggerate differences, whereas during *in situ* experiments, it is a far more realistic location within the oral environment and in which salivary flow, composition and toothbrush abrasion may make it more difficult to split differences between treatment groups.

After the agitated dietary acid challenge with grapefruit juice (on days three and four), the 8% strontium acetate dentifrice demonstrates significantly greater levels of dentine occlusion than all other products tested. The results also show that the 8% strontium

acetate is maintained on the surface of the dentine following four minutes of an agitated grapefruit juice challenge (after day four). In contrast, the alkaline agglomerate and calcium phosphate formed from the salivary amino acid arginine appear in our clinical study to be prone to dissolve following an acid challenge. The 8% strontium based dentifrice on the other hand is contained within a silica base. As such, any additional benefit this would provide in occlusion would not be expected to be affected by an acid challenge, as it is not shown to be acid labile in our clinical study and other studies conducted *in vitro* using bovine (Parkinson *et al.*, 2010; Parkinson and Willson, 2011b) and human dentine (Davies *et al.*, 2011). Two of these latter *in vitro* studies are based on a similar methodology to our clinical study, with one using dab on applications of dentifrice (Parkinson *et al.*, 2010) and another using brushing applications (Parkinson and Willson, 2011b) and support our clinical findings that dentine occlusion reduced significantly more in samples treated with 8% arginine compared to 8% strontium acetate dentifrice ($p<0.0001$) on day four following a grapefruit acid challenge. These laboratory studies describe the occlusion deposits formed by the 8% arginine-based dentifrice as acid labile. One of these studies (Parkinson and Willson, 2011b) reports a marginally significant difference in the mean score ($p<0.0786$) of 8% strontium acetate and 8% arginine on day three compared to our clinical study ($p<0.0001$) although the significance by day four was the same in all three studies ($p<0.0001$) (Parkinson *et al.*, 2010; Parkinson and Willson, 2011b). Unlike our study, the laboratory studies did not use an agitated acid challenge.

Very recently, an *in situ* study was published, which investigated 8% strontium acetate and 8% arginine dentifrice versus 1450ppm control paste following acid challenge. It used bilateral buccal appliances on 28 subjects each containing two dentine samples (Seong *et al.*, 2012). Unlike in our study, samples were only removed on two of the four treatment days for SEM imaging. Nonetheless, the results showed that both the 8%

arginine and 8% strontium acetate resulted in better dentine tubule occlusion compared to controls both with and without an acid challenge and that following an acid challenge, the 8% strontium achieved more occlusion than the 8% arginine dentifrice ($p<0.02$) (Seong *et al.*, 2012). The acid challenge in this study was the same as in our study but it did not involve agitation and could be considered a weaker challenge.

Other studies have also employed alternative erosive beverages. One laboratory study conducted *in vitro* over four days using human dentine, shows that the occlusion deposit formed by 8% arginine is acid resistant following an acid challenge using the Coca-Cola drink (Lavender *et al.*, 2010). However, only two applications of one minute agitated acid challenge were used whereas in our study the appliances were placed in the grapefruit juice twice for up to four minutes. Although the pH of many other popular beverages such as the Coca-Cola drink is lower than grapefruit juice, the latter has a higher titratable acidity and therefore greater erosive potential than Coca-Cola (Grenby *et al.*, 1989). Another laboratory study conducted using 0.3% citric acid, shows that dab on applications of an 8% arginine and strontium acetate based dentifrice to human dentine result in significant dentine tubule occlusion, and following 10s and 30s acid challenge (Davies *et al.*, 2011). Therefore, despite being more susceptible to the stronger (longer and agitated) acid challenge used in our *in situ* study, the 8% arginine based dentifrice may have use for weaker acid challenges. Its disappearance with longer 2 or 5-minute acid challenges in the *in vitro* study (Davies *et al.*, 2011) suggests it could be a surface phenomenon. In the laboratory study, the strontium acetate paste retained its level of occlusion after immersion in acid for two or five minutes compared to controls (Davies *et al.*, 2011). This *in vitro* study also included representative SEM imagery of the positive controls used in our *in situ* study. Unlike in our *in situ* study, the *in vitro* study demonstrated more of surface deposit. However, in our study, particulate deposits can be seen within and around the dentine tubules (days one to four) with the

dentine tubules becoming less apparent with each successive treatment day. This difference might be in the nature of the study type (*in vitro* vs. *in situ*), SEM imagery, or as a result of the acid challenge type (agitated vs. still).

The resistance of occlusion-based dentifrices to an acid challenge is important given today's health conscious diets. In particular, data indicate that the UK market share of erosive beverages has increased significantly over the previous decade and the most important of these are the fruit juices (BSDA, 2011), which have high titratable acidity and are strongly associated to tooth wear (Bartlett *et al.*, 2011b). Considering the strong acidic challenge used in our *in situ* study, the occlusion demonstrated by the 8% strontium acetate based dentifrice suggests a robustness of dentine tubular occlusion against many dietary erosive beverages.

Definitive conclusions could not be drawn on the presence and constituents on dentifrice deposits within the dentine tubules following EDX analysis and SEM cross sectional imaging. The desensitising dentifrices act from the surface of the dentine sample therefore the most important observation was from near the surface of each cross-sectioned sample. However, for the SEM cross-sections, the site of cross section influenced the presence or absence of un-occluded tubules. In addition, this could also affect observation of a dentine tubule throughout the image. For the EDX analysis, elementary analysis was highly variable and may be due in part to rough or inhomogeneous samples and the problems associated with over voltage (Battjes 2004). Previous work using EDX has shown that following treatment of dentine samples with 8% arginine-based dentifrice, the occluded mineral within dentine tubules reveals the presence of calcium, phosphate and carbonate (Petrou *et al.*, 2009). However, the amounts found in this *in situ* study were similar to samples treated with 8% strontium acetate and control.

Dentine samples in our study were prepared from recently extracted caries free human teeth from adults at least eighteen years of age to regulate external variables such as caries and sclerosis. They were sectioned and prepared in a similar fashion by the same member of staff to standardise dentine tubule size, distribution and orientation as far as possible. Samples were held in appliances for at least five hours on each study day and this helped achieve an oral environment with intra-oral systems including salivary composition, flow rate and influence of salivary pellicle for the dynamics of the exposed dentine surface. Variability was also reduced using one member of site staff to weigh and apply treatments across all subjects throughout the entire study and using *ex vivo* timed applications of product. The treatment was therefore standardised from the outset with respect to method used, time, toothbrush force, head and filament orientation. A split mouth, cross over design was used to allow each subject to act as their own control and to optimise model sensitivity by reducing experimental variability within each treatment group. While no carryover effect was expected between treatments either between periods or as a result of the split mouth design, a washout period of a minimum of 48 hours was incorporated and product was applied *ex vivo*. It was of note that water did not result in a grade of 4 (for the mean score) which indicated “mostly un-occluded”. This indicates that some of the dentine tubules were occluded (because all dentine samples were grade 5 or “un-occluded” at baseline). As the study had a split mouth design, it is feasible that there may have been some cross over effect and hence greater occlusion than expected in the control or water samples, due to the effect of the 8% arginine or 8% strontium dentifrice on the contra lateral side. Nonetheless, the treatments were applied *ex vivo* and excess dentifrice was removed from the appliances post brushing. Furthermore, the ANOVA analysis revealed no difference in the mean score due to treatment within each subject. An alternative would

have been for each subject to receive one treatment on each week however this would have doubled the length of the study.

Using a randomised controlled design helped negate the confounding complications such as spurious causality and bias and has been used in similar studies comparing dentine occlusion technologies (Addy *et al.*, 1987c; Claydon *et al.*, 2009). Strict inclusion/exclusion criteria also helped limit inter and intra subject variations. The brush timings were realistic of twice daily for ten seconds duration (per four dentine surfaces) as recommended in the evidence informed guidelines of oral health for the general population (DoH, 2009). In addition, the time frame for the study was realistic and all participants were able to complete the study. No acid challenge was used on days one and two in order to investigate for the first time *in situ* the dentine occlusion properties of these dentifrices. Assuming a dentine occlusion was provided, an acidic challenge was then introduced using a popular erosive beverage to investigate if the dentine occlusion remained resistant to this challenge.

In this study it was possible for the first time to directly examine a dentine sample taken from the appliance on each day following treatment and acid challenge in a non-invasive way. It is unlike previous studies, which have used a replica impression technique to visualise dentine samples (Claydon *et al.*, 2009). This allowed better visualisation of the dentine surface and SEM is a standard approach to help visualise dentine tubules post dentifrice treatment (Banfield and Addy, 2004). The ordinal occlusion scale used in this study is an uncomplicated scale, which has been used in studies previously (Claydon *et al.*, 2009; Parkinson and Willson, 2011b). Samples were not imaged and graded at day zero because at this stage, all samples were prepared with patent, cross sectioned tubules and screened using TSM imaging and SEM. Following each study day, SEM images were taken from the centre of the image by the

same member of staff who was blinded and independent from image scoring to avoid bias and ensure consistency in image preparation.

4.6 Conclusions

This *in situ* study has shown that both a strontium acetate and arginine-based dentifrice result in statistically significant dentine tubular occlusion compared to controls, using a visual ordinal scale to measure dentine tubule occlusion. The strong dietary acidic challenge (*ex vivo*) was shown to significantly impact the occlusion provided by the 8% arginine based dentifrice after its introduction on days three and four. The occlusion provided by 8% strontium acetate was not significantly impacted by the same challenge.

Chapter 5 A novel method to quantify tubule occlusion of *in situ* dentine samples

5.1 Section 1; Comparison of a novel computational and imaging method to an established visual ordinal 'standard'

5.1.1 Aim

The aim of this study was to compare an innovative, high resolution computerised method, to quantify dentine tubule occlusion of *in situ* dentine samples using SEM and TSM imaging, to an existing 'standard' (visual ordinal scale).

The visual ordinal scale will be described in this Chapter as a visual ordinal 'standard'.

5.1.2 Null hypothesis

The null hypothesis was that the automated computerised technique was unable to measure the dentine tubules and was in poor agreement with the existing 'standard' (visual ordinal scale).

5.1.3 Method

5.1.3.1 *In situ* study

This study was carried out as part of an *in situ* study to investigate the dentine tubule occlusion of dentifrices designed to treat DH. The *in situ* study received favourable ethical approval by the North West London Research Ethics Committee 11/LO/07/07. It was designed the same as the *in situ* model described in Chapter 4. A further 650 dentine samples were prepared, as described in section 2.2. From these 480 samples

were available for the *in situ* study and the remaining 140 were not used because the dentine tubules were at the incorrect orientation.

In total, 31 subjects were screened, of whom 30 were randomised and 29 completed the study. Four treatments were used and they included;

- Positive controls (both containing 5% Calcium Sodium Phosphosilicate (5% NovaMin®) and 1450ppm fluoride (as sodium monofluorophosphate));
    - Experimental dentifrice formulation employing a different surfactant and silica (higher RDA),
    - Commercial dentifrice (Sensodyne® repair and protect) (lower RDA).
- Negative controls;
    - Control dentifrice (1450ppm fluoride as sodium fluoride),
    - Volvic mineral water.

Following each day of the *in situ* study, one dentine sample was removed from each intra-oral appliance and immediately imaged using the TSM, as described in 2.2.2.6. Then, for SEM imaging, the samples were dried overnight, fixed to pin stubs and gold sputter coated post-treatment in a vacuum and imaged as described in 2.2.2.5. The TSM and SEM images were each acquired from the centre of each sample and saved in TIFF format by the operator who was blinded from the treatment groups.

5.1.3.2 Comparison of computational analysis of TSM and SEM images to the visual ordinal 'standard'

Eleven samples were lost during the study protocols. Dentine tubule occlusion was measured for 469 SEM images of dentine samples from the *in situ* study using a visual ordinal scale ('standard') by the three previously calibrated and blinded examiners as described in section 2.3. A mean score of the visual ordinal scale was then calculated

per SEM image. The visual ordinal scale was not used on the TSM images due to the poor calibration results, as shown in section 2.3.3.3.

The computer software was then run for all 469 SEM and TSM images and the outcome data provided the number of un-occluded dentine tubules per image, which provided a quantitative measurement of dentine tubule occlusion. The method for the visual ordinal scale ('standard') and computational software assessment are described in sections 2.3 and 2.4 respectively.

Reproducibility of the number of dentine tubules counted visually and by the computational assessment were conducted on 48 (10%) randomly selected SEM and TSM images from the *in situ* study and assessed using intra-class correlation. For the visual ordinal 'standard', inter-examiner agreement was assessed using plain Cohen kappa of examiner 1 vs. 2, 2 vs. 3 and 1 vs. 3 and intra-examiner agreement of the 'standard' was then assessed for each examiner by re-grading the same SEM images 3 months post study. The results from both SEM and TSM computational analyses were then correlated using Spearman's correlation coefficient.

5.1.4 Results

A total of 469 samples were collected from the study and each had a corresponding SEM and TSM image. The visual ordinal 'standard' measured the amount of dentine tubule occlusion on SEM images as a mean categorical grade, which was 3 (SE 0.1, SD 1, range 1-5). The SEM (x2000) computational analysis measured the number of un-occluded tubules greater than 0.83μm and was a mean 31 (SE 1, SD 20, range 0-121, 95% CI 30, 33). Then, the TSM (x40) computational analysis, which was also performed on the whole image from each sample, measured the number of un-

occluded tubules greater than 0.83μm and was a mean 184 (SE 3, SD 62, range 17-353, 95% CI 179, 190). Figure 48 shows an example of a SEM and TSM image and the concomitant analysis performed using software to count the number of un-occluded dentine tubules. The location of un-occluded tubules calculated by the computational analysis is also shown with the original SEM or TSM image overlapped. This shows that the position of un-occluded dentine tubules is in close proximity to the areas highlighted on each image by the software.

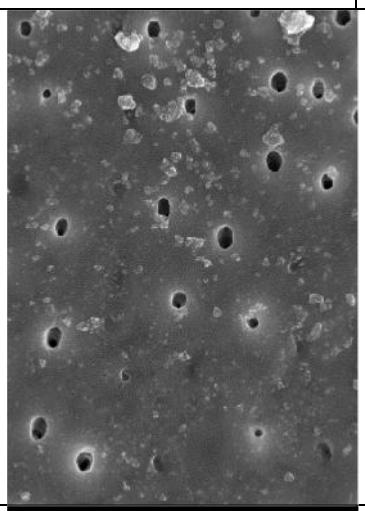
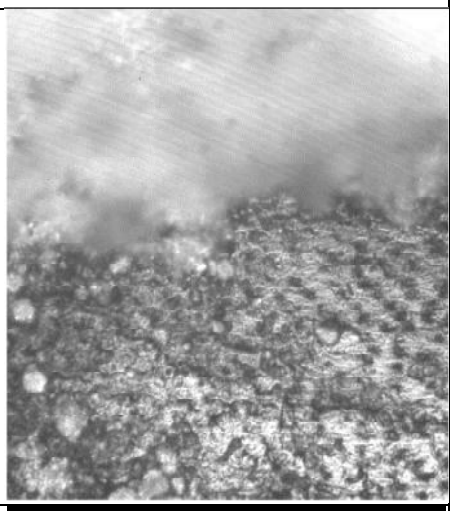
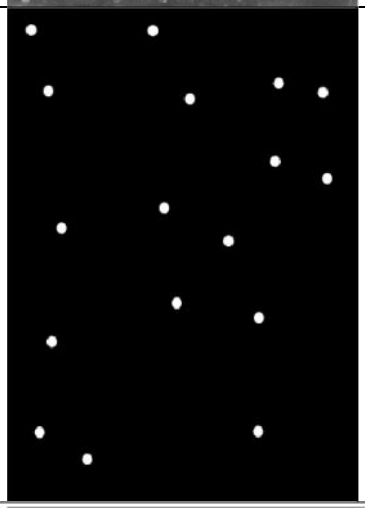
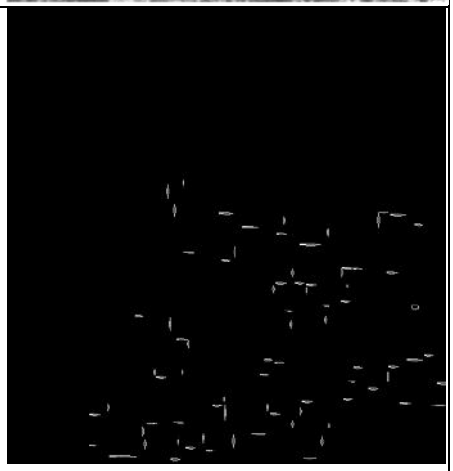
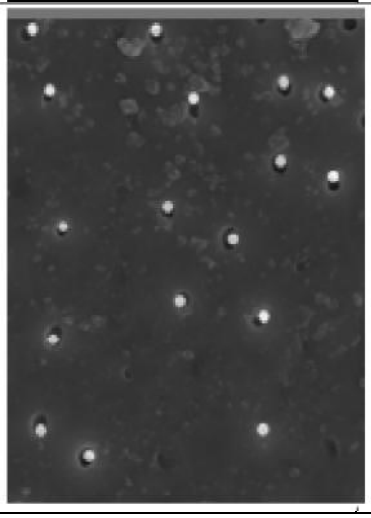
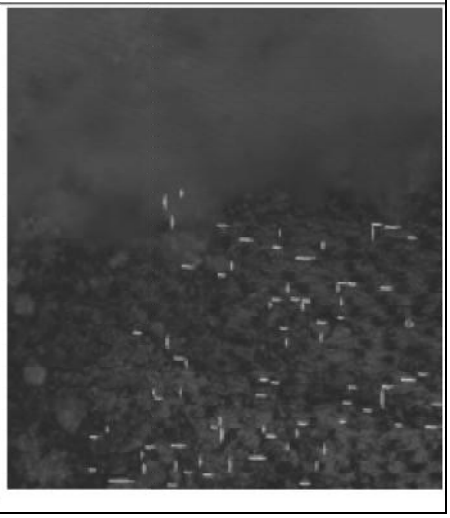
	Imaging method	
	SEM (x2000)	TSM (x40)
Original image		
Computational analysis		
Computational analysis with original image overlapped		

Figure 48 Computational analysis for SEM and TSM

Table 30 shows the mean number of un-occluded tubules calculated by the SEM and TSM computational analysis for images graded as 1-5 using the visual ordinal 'standard' for the 469 SEM and TSM images respectively. This shows that as the score for the visual ordinal scale increases, the mean number of un-occluded dentine tubules calculated by the software analysis also increases for both SEM and TSM (which were taken at different resolution). However, the difference in the number of tubules for TSM between the visual score of 4 and 5 was less than the standard error of the number of tubules for TSM at a visual score of 5. In addition, for a visual ordinal score of 1 (indicating complete occlusion), un-occluded tubules are counted using SEM and TSM computational analysis.

Table 30 Output from computational analysis for each mean visual ordinal grade

Mean visual ordinal scale	**Total number of dentine samples analysed**	**Mean number of un-occluded tubules from computational analysis (Standard error)**	
		SEM	**TSM**
5	40	57 (4)	223 (11)
4	77	41 (2)	216 (5)
3	126	33 (1)	195 (5)
2	154	23 (1)	168 (4)
1	51	18 (3)	138 (9)

Intra-class correlation of the computational analysis to a visual count of un-occluded tubules on 48 randomly chosen SEM and TSM images was >0.8. Then, validity of the visual ordinal scale was assessed on all 469 SEM images from the study. Inter- and intra-examiner reproducibility of the visual ordinal 'standard' using plain kappa for each

of the three examiners was 0.3-0.5 and 0.3-0.5 respectively, as shown in Table 31.

Table 31 Inter- and Intra- examiner reproducibility of the visual ordinal 'standard'

Examiner	**Inter-examiner plain kappa agreement**
Examiner 1 vs. 2	0.3
Examiner 2 vs. 3	0.4
Examiner 1 vs. 3	0.5
Examiner	**Intra-examiner plain kappa agreement**
Examiner 1	0.3
Examiner 2	0.4
Examiner 3	0.5

The Spearman correlation of the visual ordinal ‘standard’ used to grade SEM images compared to the number of un-occluded tubules counted using a computational analyses for SEM was +0.58 ($p<0.001$) on 469 images. Then, Spearman correlation of the visual ordinal standard to the number of un-occluded dentine tubules counted using computational analysis of an entirely different sample processing and imaging technique (TSM) was +0.42 ($p<0.001$) on 469 images.

5.1.5 Discussion

The novel computational and imaging routine using SEM and TSM was described in Chapter 2. In this Chapter, the technique has been applied to dentine samples collected from an *in situ* randomised clinical study to investigate dentine tubule occlusion and the data shows that it is capable of analysing and counting tubule occlusion quantitatively in dentine samples. It also has positive associations with the established technique for measuring dentine tubule occlusion using the visual ordinal

'standard' ($p<0.001$). As the visual ordinal 'standard' increases, the mean output from the SEM and TSM computational analysis also increases, indicating more un-occluded dentine tubules. Interestingly, the SEM and TSM computational analysis also count un-occluded dentine tubules in samples, which were graded as occluded (grade 1) using the visual ordinal scale.

Intra-class agreement of the computational analysis to the numbers of un-occluded dentine tubules counted visually from a randomly chosen 10% of the sample were ≥ 0.8 for SEM and TSM images. Previous *in vitro* work demonstrates similar high correlations using SEM (Ciocca *et al.*, 2007). Computational analysis routines, unlike manual processes such as visual counting and the visual ordinal 'standard', are fully automated, permit identical interpretation of images, remove subjectivity and make the measurement process reproducible and precise. Manual processes have been shown to increase variability when measuring the amount of un-occluded dentine tubules (Ahmed *et al.*, 2005). Indeed, the inter- and intra-examiner agreement from all samples was 0.3-0.5 for the visual ordinal 'standard' hence our reluctance to call this a gold standard. Furthermore, unlike visual interpretation, computational analysis removed the need for prolonged inspection of each image and provided analysis of each image at a higher resolution by processing individual pixels in each image. In contrast, visual interpretation of the TSM images (taken at x40) was more difficult than the SEM images (taken at x2000). This is reflected in a poor intra-examiner agreement using the visual ordinal 'standard' to measure TSM (≤ 0.3) compared to SEM (>0.7) images during calibration in Chapter 2.

The computational analyses, unlike the visual ordinal 'standard', measured dentine tubules greater than 0.83μm diameters, which were reported to be a clinically meaningful diameter leading to measurable DH (Absi *et al.*, 1987). Previous work

discussed that dentine tubule recorded as a maximum diameter is independent from tubule orientation (Arends *et al.*, 1995; Schilke *et al.*, 2000), which is particularly relevant considering that variation in the size of untreated tubules and their density throughout the tooth is highly varied (Mjor and Nordahl, 1996). Studies using computational assessments have measured the area of un-occluded dentine tubules on SEM images taken post treatment only (Banfield and Addy, 2004; Ciocca *et al.*, 2007; Lee *et al.*, 2008). However, it has been shown that the total area of un-occluded tubules is affected significantly by dentine tubule orientation (Ahmed *et al.*, 2005) and could therefore lead to erroneous results if images are not taken of the same sample pre- and post-treatment to allow paired comparisons to be made. The use of a maximum diameter as a cut off for DH also echoes the importance of reducing tubule diameter in DH management and not simply creating complete occlusion (Markowitz and Pashley, 2008).

The SEM computational analysis correlated slightly more than the TSM computational analysis to the visual ordinal 'standard'. This was probably because the 'standard' and SEM computational analyses both used the same image. In contrast, TSM used a different image but had the advantage that it did not involve sample dehydration or gold sputter coating and was a minimally invasive technique. This is important considering the diameter of tubules has been shown to decrease significantly following dehydration (Arends *et al.*, 1995). The TSM was taken at a lower resolution (x40) than the SEM (x2000). Whereas most previous dentine tubule occlusion studies have used SEM, the TSM is a novel imaging technqiue for *in situ* analysis of dentine samples and tubule occlusion and has not been compared with an established method of measurement to date.

5.1.6 Conclusion

In conclusion, the computational analysis for the SEM and TSM are accurate and correlate statistically significantly with the visual ordinal 'standard'. Correlations of the visual ordinal scale to the TSM computational analysis were less, probably due to differences in image processing techniques, but the TSM requires minimal sample preparation compared with SEM.

5.2 Results by treatment using established and novel measurement techniques

5.2.1 Aim

The aim of this study was to investigate the dentine occlusion and acid resistance of an experimental dentifrice developed to treat DH after 4 days of twice daily brushing with an acid challenge on days three and four. This was undertaken in conjunction with the *in situ* study conducted in section one of this chapter. The results of the established visual ordinal scale are presented in addition to the novel imaging and computational analysis methods.

5.2.2 Null hypothesis

The null hypotheses were that the experimental dentifrice did not occlude the dentine tubules and was soluble in acid in comparison with other treatments using the visual ordinal standard and the computational analysis methods.

5.2.3 Method

The methodology was discussed in section one of this chapter. The remainder of this chapter will discuss the results of each treatment using the visual ordinal scale and the novel computational and imaging routines. Unlike the SEM, images were also taken of the samples using TSM before treatment and therefore these results are also included. TSM images were taken of the samples before each treatment in the same way as post treatment, using the protocol previous described in section 2.2.2.6. The results of each image measured using the visual ordinal 'standard', SEM computational analysis and the TSM computational analysis, were standardised using an equation. This was to enable interpretation of the results from each measurement technique. The formula used was;

$$\text{Standardised value} = \frac{\text{Actual value - Mean of that group}}{\text{Standard deviation for that group}}$$

5.2.4 Results

Of the thirty subjects who were randomised, twelve subjects (40.0%) were male and eighteen subjects (60.0%) were female with a mean age of 35.3 years (SD=8.75); eighteen were white (60.0%), six black or African American (20.0%) and six Asian (20.0%). Review prior to un-blinding identified six protocol deviations with the potential to affect efficacy recorded across six subjects, which each led to exclusion of their specific data only on specific treatment days. For example, administering the wrong treatment on an appliance, or the dosing time for a treatment was not recorded.

Statistical analysis using ANOVA found no difference in the visual ordinal 'standard', SEM and TSM computational analyses due to period of the study (week one or two), side of mouth or subject. Differences in the visual ordinal standard, SEM and TSM computational analyses did occur for day and treatment.

The TSM computational analysis showed that for all 469 samples, the mean number of un-occluded dentine tubules before the *in situ* study at baseline (day zero) was 222 (SE 2, SD 41, 95% CI 218, 226). Then, post treatment the mean number of un-occluded tubules for all 469 samples (treated with various treatments) was 184 (SE 3, SD 62, 95% CI 179, 190). This difference was statistically significant ($P<0.0001$).

Standardised outcomes and standard errors for treatment and day are shown for the visual ordinal 'standard', the TSM computational analysis and SEM computational analyses in Figure 49. Lower scores indicate that the dentine tubules are more occluded. The order of treatments (ranked by highest to lowest occlusion) for 'standard' and TSM computational analysis on days one to four and for SEM computational analysis on day four were calcium sodium phosphosilicate high RDA (experimental dentifrice) >calcium sodium phosphosilicate low RDA (Sensodyne Repair and Protect® commercial dentifrice) >control paste >water. For the TSM, there was a statistically significant increase in the amount of dentine tubule occlusion between day 0 (baseline) and day one for the experimental dentifrice ($p<0.0001$). Following acid challenge (days three and four), there were also statistically significant decreases in dentine tubule occlusion between day two and day three for the visual ordinal 'standard' and SEM computational analysis for water ($p<0.0001$). The SEM computational analysis showed that the occlusion for the experimental dentifrice on days one to three was higher than the commercial dentifrice. This was not statistically significant.

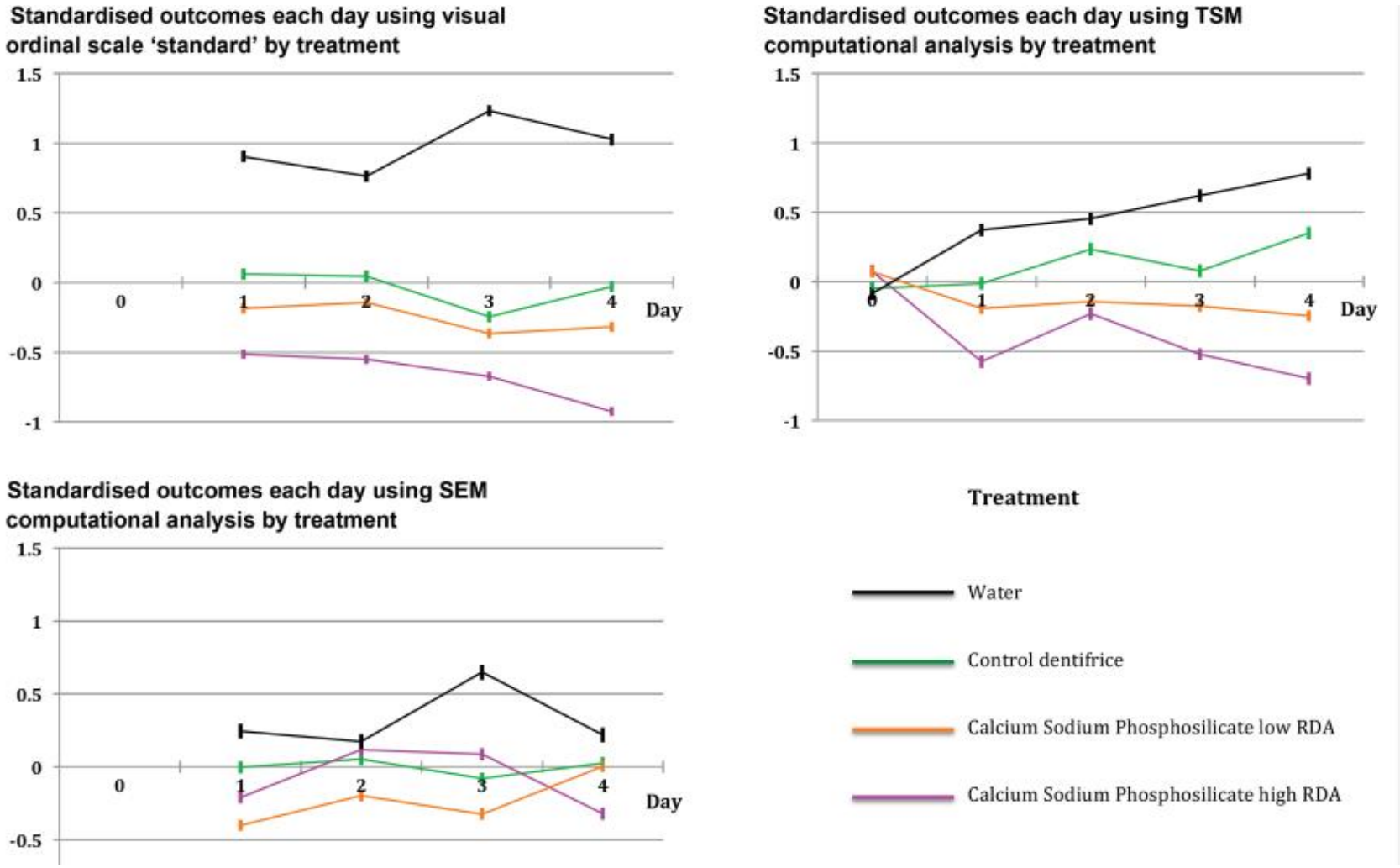


Figure 49 Standardised outcomes and standard errors for treatment and day are shown for the 'standard' (top left), the TSM computational analysis (top right) and SEM computational analyses (bottom left).

Table 32, Table 33 and Table 34 show the difference in standardised scores between experimental and other treatments for the visual ordinal scale, SEM computational analysis and TSM computational analysis respectively. The visual ordinal 'standard' and TSM computational analysis both showed similar statistically significant differences in the number of un-occluded dentine tubules between the experimental dentifrice and water ($p \leq 0.007$) on days one to four of the *in situ* study. In addition, by day four, the visual ordinal 'standard' and TSM computational analysis both showed statistically significant differences in the number of un-occluded dentine tubules between the experimental dentifrice and control ($p<0.001$).

The visual ordinal 'standard also showed statistically significant differences in the number of un-occluded dentine tubules between the experimental dentifrice and control paste on days one and two and the experimental dentifrice and commercial dentifrice on days one and four ($p<0.05$). The TSM computational analysis did not show these statistically significant differences. The SEM computational analysis did not show statistically significant differences between the experimental dentifrice and other treatments on days one to four.

Table 32 Differences in the standardised mean visual ordinal grade and associated 95% confidence intervals and p-values from ANOVA model including subject as a random effect, and treatment, period and location of sample in mouth (left or right) as fixed effects. Differences are first named treatment minus second named treatment such that a negative difference favours the first named treatment. An acid challenge occurred days three and four.

Day	Comparison of treatments	Difference	95% CI	P Value
1	Experimental paste vs. Water*	-1.42	-1.99, -0.84	<0.0001
	Experimental paste vs. Control paste*	-0.57	-1.14, -0.011	0.044
	Experimental paste vs. Commercial dentifrice*	-0.33	-0.90, 0.24	0.044
2	Experimental paste vs. Water*	-1.31	-1.91, -0.72	<0.0001
	Experimental paste vs. Control paste*	-0.60	-1.19, 0.00	0.048
	Experimental paste vs. Commercial dentifrice	-0.41	-1.00, 0.19	0.281
3	Experimental paste vs. Water*	-1.90	-2.43, -1.37	<0.0001
	Experimental paste vs. Control paste	-0.43	-0.95, 0.10	0.149
	Experimental paste vs. Commercial dentifrice	-0.31	-0.83, 0.22	0.429
4	Experimental paste vs. Water*	-1.95	-2.47, -1.43	<0.0001
	Experimental paste vs. Control paste*	-0.90	-1.42, -0.37	<0.0001
	Experimental paste vs. Commercial dentifrice*	-0.61	-1.14, -0.08	0.019

Table 33 Differences in the standardised SEM computational analysis and associated 95% confidence intervals and p-values from ANOVA model including subject as a random effect, and treatment, period and location of sample in mouth (left or right) as fixed effects. Differences are first named treatment minus second named treatment such that a negative difference favours the first named treatment. An acid challenge occurred days three and four.

Day	Comparison of treatments	Difference	95% CI	P Value
1	Experimental paste vs. Water	-0.46	-1.04, 0.13	0.178
	Experimental paste vs. Control paste	-0.21	-0.78, 0.36	0.776
	Experimental paste vs. Commercial dentifrice	0.19	-0.39, 0.77	0.824
2	Experimental paste vs. Water	-0.06	-0.76, 0.64	0.996
	Experimental paste vs. Control paste	0.06	-0.64, 0.76	0.995
	Experimental paste vs. Commercial dentifrice	0.31	-0.38, 1.01	0.642
3	Experimental paste vs. Water	-0.56	-1.32, 0.20	0.218
	Experimental paste vs. Control paste	0.17	-0.58, 0.91	0.937
	Experimental paste vs. Commercial dentifrice	0.41	-0.34, 1.16	0.486
4	Experimental paste vs. Water	-0.54	-1.15, 0.07	0.099
	Experimental paste vs. Control paste	-0.35	-0.96, 0.26	0.443
	Experimental paste vs. Commercial dentifrice	-0.33	-0.94, 0.29	0.518

Table 34 Differences in the standardised TSM computational analysis and associated 95% confidence intervals and p-values from ANOVA model including subject as a random effect, and treatment, period and location of sample in mouth (left or right) as fixed effects. Differences are first named treatment minus second named treatment such that a negative difference favours the first named treatment. An acid challenge occurred days three and four.

Day	Comparison of treatments	Difference	95% CI	P Value
1	Experimental paste vs. Water*	-0.95	-1.53, -0.37	<0.0001
	Experimental paste vs. Control paste	-0.56	-1.13, 0.00	0.052
	Experimental paste vs. Commercial dentifrice	-0.38	-0.95, 0.18	0.293
2	Experimental paste vs. Water*	-0.69	-1.23, 0.14	0.007
	Experimental paste vs. Control paste	-0.47	-1.01, 0.08	0.119
	Experimental paste vs. Commercial dentifrice	-0.90	0.63, 0.45	0.972
3	Experimental paste vs. Water*	-1.14	-1.79, -0.50	<0.0001
	Experimental paste vs. Control paste	-0.60	-1.24, 0.04	0.075
	Experimental paste vs. Commercial dentifrice	-0.35	-0.99, 0.30	0.499
4	Experimental paste vs. Water*	-1.48	-2.01, -0.94	<0.0001
	Experimental paste vs. Control paste*	-1.05	-1.58, -0.52	<0.0001
	Experimental paste vs. Commercial dentifrice	-0.45	-0.98, 0.08	0.121

5.2.5 Discussion

This study has shown that after four days of twice daily brushing with an acid challenge on days three and four, the experimental dentifrice showed statistically significantly more dentine occlusion than the water and control paste using the visual ordinal 'standard' and TSM computational analysis assessment ($p<0.0001$). The experimental dentifrice also showed statistically significantly more dentine occlusion on day four compared to the commercial dentifrice for the visual ordinal standard only ($p=0.019$). The SEM computational analysis did not show any statistically significant between treatment differences.

The computational analyses for TSM and SEM, unlike the visual ordinal 'standard' using the SEM, showed less significant differences between the experimental dentifrice and other treatments. This may be because the computational analysis identifies and excludes all dentine tubules less than 0.83μm diameter from the analysis. In contrast, the visual ordinal 'standard' does not differentiate from these un-occluded dentine tubules. In the results, it was shown that some un-occluded dentine tubules (greater than 0.83μm) were still present using the computational analysis even in complete occlusion (grade 1) using the visual ordinal scale. It is expected that this minimum diameter is clinically relevant in DH (Absi *et al.*, 1987). Hence, DH could potentially have been present in cases visually assessed as completely occluded using the visual ordinal 'standard'.

The computational analysis for the SEM showed no statistically significant differences between the experimental dentifrice and other treatments. This might indicate that there was no difference in the number of un-occluded dentine tubules between the various treatments. In addition, perhaps the shrinkage that occurred during processing

for SEM might have excluded more dentine tubules from the SEM computational analysis than the visual ordinal scoring or TSM computational analysis.

The SEM computational analysis showed that the dentine occlusion was more (but not statistically significant) for the experimental control group compared to the commercial dentifrice on day four, but not on days one to three (unlike the visual ordinal 'standard' and TSM computational analysis). This might be explained by the surface particulate deposits for the experimental treatment, which were larger compared to the other treatments and largest on the SEM compared to TSM images. They were anatomically difficult for the software to differentiate. The acid challenge on days three and four involved agitation and removal of these large deposits and might help explain why the standardised score for the visual ordinal scale, SEM and TSM computational analyses was lowest (and dentine tubule occlusion was greater) for the experimental dentifrice compared to the other treatments by day 4.

Chapter 6 Discussion

6.1 General discussion

The overall theme of this thesis is the role of acids in DH and tooth wear. The first studies investigated the prevalence of tooth wear and DH in a convenience sample (n=350) of 18-33 year old patients. The following studies investigated the effect of an acid on tubule occlusion of dentifrices and finally the occlusion of a new dentifrice. The prevalence results indicated that 91% of subjects had tooth wear (BEWE score of 1 and above) and 43% had DH (Schiff score of 1 and above) and the results from tooth wear and DH using the BEWE and Schiff sextant cumulative scores respectively were correlated ($p<0.0001$). Both dentifrices designed to occlude dentine tubules (containing 8% strontium acetate and 8% arginine respectively) were successful and the first also showed resistance to *ex vivo* acid immersion. In addition new assessment procedures were evaluated to record tooth wear and DH and to count the number of tubules occluded in an *in situ* study.

A BEWE and Schiff score taken as a sextant cumulative score from each subject provided a measurement of the tooth wear and DH occurring on each subject. These results could then be compared to aetiological factors recorded from a subject questionnaire in Chapter 3. This was the first study to date investigating tooth wear and DH on all tooth surfaces with associated aetiological factors. The result showed that as the severity of tooth wear increased, the severity of DH was also likely to increase ($p<0.0001$). Subjects who reported consuming acidic foods “often”, or in close proximity to brushing were more likely to report DH. However, surfaces with tooth wear did not necessarily have DH. Also, subjects who reported having DH in the previous twelve

months were not likely to also report DH currently or have DH at the clinical appointment. This suggests that DH is not always present and supports an episodic nature of DH. These results support the main process described in the aetiology of DH. This necessitates that dentine is exposed by tooth wear or gingival recession (lesion localisation) and secondly that the dentine tubular system is patent from the surface of the tooth to the pulp (Addy, 2002). Many studies have focused on sensitivity affecting the cervical or buccal tooth surfaces. In this study DH was observed to be associated with wear on the occlusal tooth surfaces of teeth. These surfaces are likely to also have attrition as well as erosion and abrasion acting and would be expected to also have DH. Clinical trials are often used to investigate the potential of desensitising dentifrices and measure DH on patients directly. However, they rely on recruitment of hundreds or even thousands of subjects who have DH at the start of the study (Holland *et al.*, 1997). Therefore it is very challenging to standardise the presence of DH and other aetiological factors at the beginning of the study.

An alternative to clinical trials is to measure dentine tubule occlusion of dentine samples. This requires excellent standardised sample preparation procedures. Ideally, the studies on dentine tubule occlusion would be conducted *in vivo* but there are no imaging techniques with sufficient resolution and which allow sufficient stabilisation of the sample to allow observations of dentine tubules. *In situ* studies therefore provide advantages of both a clinical study and surrogate investigation of a dentine sample. Prevention and management strategies in DH require occlusion of dentine tubules and hence accurate measurement of tubular occlusion is important. The *in situ* study in Chapter 4 used an established visual ordinal scale (or 'standard') to measure dentine tubule occlusion. The *in situ* study was designed with a stronger acid challenge than had been used in previous *in situ* studies investigating dentine tubule occlusion to date, using an *ex vivo* agitated grapefruit juice challenge. The results showed that the 8%

arginine and 8% strontium based dentifrice resulted in dentine tubule occlusion, but that the 8% arginine based dentifrice was susceptible to an acid challenge and this was statistically significant. Brushing alone with water in this *in situ* did not impact on dentine tubule occlusion statistically significantly, but interestingly the treatment group for water was not fully un-occluded (grade 5 using the visual ordinal scale). In conclusion, dentine tubule occluding agents that offer acid resistant properties have potential in the management of DH due to the importance of erosion in its aetiology (Markowitz and Pashley, 2008).

The visual ordinal scale provided categorical information on the amount of dentine tubule occlusion, but a computational assessment was developed and validated for Chapter 5 to quantify dentine tubule occlusion. This provided more information than the visual ordinal scale and it was accurate compared to a visual count of the number of un-occluded tubules. It also included a cut off for minimum diameter and reflects the importance of reduction in tubule diameter and not simply complete occlusion in reducing DH. Moreover, using a minimum diameter is less affected by changes in the orientation of the dentine sample than area, which was reported in previous studies. The computational analysis was compared to a visual method of measuring dentine tubule occlusion and showed reasonable correlation using SEM images. Also, for the first time in an *in situ* study, TSM was used to image the surface of dentine samples and used in a computational analysis routine. The computational analysis was then able to quantify the number of un-occluded dentine tubules on each image. Imaging before and after treatment enabled variations in each dentine sample to be accounted and observe differences in dentine tubule occlusion after just one days brushing. The SEM computational analysis did not find any statistically significant differences between the experimental dentifrice and other treatments by day four of the *in situ* study. In contrast, the visual ordinal scale for the SEM and the computational analysis

for the TSM showed that the experimental dentifrice resulted in statistically significantly more occlusion than the other treatments by day four. The TSM, unlike the SEM, required minimum sample processing in order to obtain an image of the surface of the dentine sample and could be considered the least destructive imaging technique. The various processing, imaging and assessment techniques used in studies on dentine tubule occlusion have been shown to produce quite different results and studies should therefore be compared with caution.

6.2 Conclusions

Referring back to the aims of Chapters 2, 3, 4 and 5, it can be concluded that:

Chapter 2

- The BEWE and Schiff sextant cumulative scores are suitable to measure tooth wear and DH respectively per subject,
- The visual ordinal scale can be calibrated to categorise the amount of dentine tubule occlusion in SEM, but not TSM images taken of dentine samples,
- A computerised method has been developed to quantify dentine tubule occlusion (based on minimum diameter of un-occluded dentine tubules) on SEM and TSM images taken of dentine samples,
- Using an *ex vivo* agitated acid challenge with grapefruit juice containing citric acid resulted in a strong acid challenge to dentine.

Chapter 3

- In a prevalence study with a convenience sample of 350 subjects, as the severity of tooth wear increases, so too did the severity of DH recorded clinically ($p<0.0001$),

- There were no statistically significant associations between tooth wear, DH and all the various aetiologies respectively, in agreement with the null hypothesis. However, more DH was observed in this sample of subjects (n=350) with increasing frequency or recent consumption of acidic food or drink.

Chapter 4

- Both a strontium acetate and arginine-based dentifrice resulted in statistically significant dentine tubular occlusion *in situ* compared to controls after two days of twice daily brushing, using a standard visual ordinal scale, which had been calibrated to measure dentine tubule occlusion. The strong dietary acidic challenge was shown to significantly impact the occlusion provided by the 8% arginine based dentifrice after its introduction on days three and four. The occlusion provided by 8% strontium acetate was not significantly impacted by the same challenge.
- This chapter has further established the visual ordinal scale as a tool to measure dentine tubule occlusion.

Chapter 5

- The computational analysis was accurate in measuring dentine tubule occlusion, correlated statistically significantly to the visual ordinal scale and provided more information than the visual ordinal standard,
- The visual ordinal 'standard' and TSM computational analysis used to measure dentine tubule occlusion both showed that an experimental dentifrice containing 5% calcium sodium phosphosilicate showed statically significantly more occlusion than the water and control paste after 4 days of twice daily brushing with an acid challenge on days 3 and 4 using both the visual ($p<0.0001$). A similar computational analysis for SEM did not show statistically significant differences. The TSM, unlike the SEM, involved minimal sample processing.

In conclusion, the overall null hypothesis of this thesis is not supported and these studies therefore support an association of DH and tooth wear.

6.3 Clinical implications

Oral health has been defined as '...the state of the mouth and associated structures where no disease exists, future disease is inhibited' whereby 'the occlusion is sufficient to masticate food and the (appearance of the) teeth are of a socially acceptable standard' (Yewe-Dyer, 1993). DH is not classified as a disease by WHO, but it has been described as one. To recapitulate, DH 'is characterised by short sharp pain arising from exposed dentine in response to stimuli typically thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other form of defect or disease'. It is evident from this definition that DH requires excellent skills in differential diagnosis to exclude other causes with similar symptoms. Patients often report to their dentist complaining of DH, but it may not always be apparent to the patient why these symptoms have occurred and they may simply want relief of the symptoms.

This thesis has recognised the importance of tooth wear and in particular erosion in the aetiology of DH. Tooth wear (attrition, abrasion and erosion), unlike DH, are diseases recognised by WHO and therefore the presence of DH could be an important diagnostic tool of a disease process. The UK population is projected to continue ageing with the average (median) age rising from 39.7 years in 2010 to 39.9 years in 2020 and 42.2 by 2035. In addition, the population size is projected to increase by 4.9 million from an estimated 62.3 million in 2010 to 67.2 million by 2020 and to 73.2 million over the 25 year period to mid-2035. The numbers of 'older people' (classified as those over 65 years of age) will increase the fastest (Statistical Bulletin: 2010-based National

population projections, 23 November 2011) and for this section of the population it is estimated that by 2028 total tooth loss will be eliminated in those under 65 years and significantly reduced in those under 75 years of age (Kelly *et al.*, 1998). The prevalence study of this thesis investigated subjects aged 18-35, attending for routine treatment in primary (63%) or secondary (37%) dental care and the prevalence of DH was relatively high (43%). However, recent research in the community (in China) suggests that DH may also be very prevalent in older subjects (Que *et al.*, 2010b). With an aging population in the UK retaining their teeth for longer and considering tooth wear, in particular erosion, has been described as increasing with age (Van't Spijker *et al.*, 2009; Steele and O'Sullivan 2011) and given the wide consumption of soft drinks in the UK, it might suggest a change in oral health trends and increase in the prevalence of tooth wear and DH in the future.

DH has been shown in this thesis to impact on lifestyle factors and social interaction and is not always described simply as pain by subjects. Over half of the subjects who had DH reported it to be important or very important and in those who had reported DH to have occurred for a number of years. Recent research has supported these findings. It has shown that DH has a big effect on everyday life activities and the features reported by patients in this research are suggestive of DH being a chronic illness in which those affected have to adjust and to integrate their disease into everyday life (Gibson *et al.*, 2010). One subject even reported in this research that "*You never forget that you have it because the decisions you make and the way you do things is affected by it*". In the study, the emotional impact of DH included guilt through not giving enough attention to their oral health and annoyance of not being able to eat foods on certain teeth or brush certain teeth. The emotional impact contributed to the pain, its unpredictability and the detrimental effects of eating and drinking. It also affected social activities and caused embarrassment, for example, one may be at a restaurant with

friends and would need to wait if food is initially too hot or cold to eat (Gibson *et al.*, 2010). Older people of today have higher expectations of oral health (Walls and Steele, 2001) and in the Gibson *et al.* 2010 study, those subjects who experienced DH for longer were more likely to describe their DH as an illness. Effective diagnosis and management of the condition could therefore save these patients considerable worry.

Effective diagnosis of DH and treatment of the condition, by first removing aetiological factors linked to tooth wear, is necessary to reduce its impact psychologically to the patient and to the tooth. Therefore, patients presenting with DH should be asked questions related to dietary habits, heartburn and vomiting and brushing habits (especially in relation to consumption of acidic food and drinks). These causative factors require prevention as a first line treatment. For example, delaying brushing after acidic food or drink in order to help prevent recurrence of the condition and exacerbating the tooth wear process. In addition, desensitising dentifrices, which occlude the dentine tubules and are resistant to acid challenges, such as 8% strontium containing dentifrice, are available commercially. These are easily applied to teeth by patients at home. An 8% strontium dentifrice is shown to have resistance to a strong acid challenge and therefore suitable for prevention of DH even in those patients with high frequency of consumption of a range of acidic food or drinks.

This thesis has focused on management of DH from the reduction in aetiological factors and use of topical desensitising agents to the exposed dentine. The author is also mindful of other products or actives, such as fluoride, which may also offer preventive roles in the management of DH, by protecting enamel and thus preventing localisation of dentine lesions. Most subjects in the prevalence study in Chapter 3 reported using fluoride toothpaste, but no statistically significant associations were shown between levels of tooth wear or DH and the use of fluoride. However, one *in*

vitro study showed that increasing concentrations of fluoride to 5, 000ppm and 19, 000ppm afforded protection to enamel from erosion and attrition, but no protective effect was shown for dentine (Austin *et al.*, 2010). A recent *in situ* study using enamel treated with periodic applications of 5000ppm fluoride showed improved resistance to enamel erosion caused by orange juice (Ren *et al.*, 2011), which could therefore aid in the prevention of dentine lesions. Such concentrations of fluoride would need to be applied to teeth professionally.

6.4 Future directions

- Translate the accurate DH measurement techniques for use in further *in vitro* or clinical (*in situ* or *in vivo*) studies to investigate the aetiological factors involved in DH (erosion, abrasion and attrition),
- Translate the accurate DH measurement techniques for use in further *in vitro* or clinical (*in situ* or *in vivo*) studies to investigate the dentine tubule occluding and acid resistance of health care designed to treat DH,
- Develop and utilise a range of microscopic techniques for the measurement of dentine tubule occlusion,
- Develop and utilise a range of image analysis software for the measurement of dentine tubule occlusion,
- Develop and utilise methods to measure DH *in vivo*.

Chapter 7 Appendices

7.1 Appendix 1 Subject questionnaire for tooth wear and dentine hypersensitivity

QB1. How many times per day do you brush your teeth? Never O Once a day O Twice a day O Three times a day

QB2. Which kind of toothbrush do you use frequently? *(Please tick one box only)*

None O Manual toothbrush O Electric toothbrush O

QB3. Which motions do you use while brushing your teeth? *(Please tick one box only)*

Various motion *O* Horizontal motion (= "Back and forth movement") O

Vertical motion (= up and down movement) O Circular motion O Don't know/ Note sure O

QB4 . How often do you brush your teeth? *(One answer per line)*

	Often	Occasionally	Rarely	Never	Don't know
After your breakfast	O	O	O	O	O
Before your breakfast	O	O	O	O	O
After lunch	O	O	O	O	O
After dinner	O	O	O	O	O

QB5. How long do you wait before brushing teeth after having your breakfast?

(Please indicate estimated average) Number of minutes.....

QB6. Are you left-handed or right-handed? *(Please tick one box only)* Left-handed O Right-handed O

QB7. How often during the 12 past months have you….? *(One answer per line)*

	Often	Occasionally	Rarely	Never	Don't know
Experience toothache due to sensitive teeth	O	O	O	O	O
Suffer from heartburn/reflux/regurgitation	O	O	O	O	O
Suffer from repeated vomiting	O	O	O	O	O
Experienced difficulties with eating food due to mouth or teeth problems	O	O	O	O	O
Felt embarrassed because of the appearance of your teeth	O	O	O	O	O
Felt tense because of teeth or mouth problems	O	O	O	O	O
Avoided conversation because of the appearance of your teeth or dentures	O	O	O	O	O

QB8. Do you consider yourself currently suffering from sensitive teeth? Yes O No O Don't know/Not sure O

If "Yes" please answer QB9, QB10 et QB11; if "No" or "Don't know/Not sure" please go directly to QB12

QB9.When does the pain from the sensitive teeth occur? *(One answer per line)*

	Often	Occasionally	Rarely	Never	Don't know
While brushing teeth	O	O	O	O	O
Cold weather (air)	O	O	O	O	O
Touch	O	O	O	O	O
Hot water	O	O	O	O	O
Sweet	O	O	O	O	O
Cold (drink, ice….)	O	O	O	O	O
Others	O	O	O	O	O

QB10. How long have you been suffering from sensitive teeth?

Less than a year *O* 1 to 2 years *O* 2 to 5 years *O* 5 or more years *O* Don't know *O* Never *O*

QB11. How would you evaluate the pain intensity of your sensitive teeth? *(Please tick one box only))*

Not important O Little importance O Some importance O Important O Very important O Don't know O

QB12. How often do you? (One answer per line)

	Often	Occasionally	Rarely	Never	Don't know
Snore	O	O	O	O	O
Take sleeping medication/antidepressants	O	O	O	O	O
Smoke cigarettes	O	O	O	O	O
Chew gum	O	O	O	O	O
Have acidic foods (i.e. fruit, fruit juice…)	O	O	O	O	O

QB13. How many eating/drinking occasions do you have per day even in small quantities?

Number of times .....

QB14. How often do you eat or drink of the following, even in small quantities? *(One answer per line)*

	Often	Occasionally	Rarely	Never	Don't know
Fresh fruit e.g lemon, orange, apple, pear, grapes, etc	O	O	O	O	O
Fruit and vegetable juice e.g. orange, apple, grapes, pineapple, carrot, multivitamin, etc.	O	O	O	O	O
Isotonic drinks/Energy drinks e.g. Isostar, Red-bull, powerade, perform, red horse etc.	O	O	O	O	O
Soft drinks i.e. Cola beverages, Lemonade, Iced tea, sprite, fanta etc.	O	O	O	O	O
Cheese, yoghurts, other dairy products	O	O	O	O	O

If at least one of « often » cases is filled please go to QB15. If not go to directly QB16

QB15. Please can you precise for each items how often do you eat or drink of the following even in small quantities? *(One answer per line)*

	More than 3 times per day	2-3 times per day	Once per day	Less than once per day but at least once per week	Less than once per week
Fresh fruit e.g. lemon, orange, apple, pear, grapes, etc.	O	O	O	O	O
Fruit and vegetable juice e.g. orange, apple, grape, pineapple, carrot, multivitamin, etc.	O	O	O	O	O
Isotonic drinks/Energy drinks : Isostar, Red-bull, etc.	O	O	O	O	O
Soft drinks i.e Cola beverages, Sprite, Lemonade, iced tea, etc.	O	O	O	O	O
Cheese, yoghurts, other dairy products	O	O	O	O	O

QB16. When did you last visit a dentist? *(Please tick one box only)*

Less than one year *O* 1 to 2 years *O* 2 to 5 years *O* 5 and more years *O* Never *O* Don't know *O*

QB17. About how many times in the 12 past mouths have you seen a dentist? ! __ !__ !

QB18. What was the reason for the last visit to the dentists? *(Please tick one box only)*

Check-up, examination or cleaning *O* Routine treatment *O* Emergency treatment *O*

QB19. How tall are you? (in cm)? .....

QB20. How much do you weigh (in kg)? .....

QB21. How often do you exercise or play sport? *(Please tick one box only)*

5 times a week or more O 3 to 4 times a week O 1 to 2 times a week O 1 to 3 times a month O Don't know O

less often O Never O

QB22. Did you wear an orthodontic appliance? Yes O No O

QB23. Do you use a toothpaste containing fluoride? Yes O No O Don't Know/Not sure O

QB24. Do you use fluoride in any other way that toothpaste? Yes O No O Don't know/Not sure O

Q25. Name of tooth paste which you currently use .....

Q26. Last time you consumed food in Q14? .....

DATE .....Site code.....Subject ID.....

7.2 Appendix 2 Clinical questionnaire for DH and tooth wear

MAXILLA	Bleeding	Buccal														
		Palatal														
	Periodontal Probing depth (mm)	Buccal														
		Palatal														
	Recession (mm)	Buccal														
		Palatal														
	BEWE code	Buccal														
	BEWE code	occlusal														
	BEWE code	Palatal														
	Schiff index/DH index	palatal														
	Schiff Index/DH Index	occlusal														
	Schiff Index/DH Index	Buccal														
			17	16	15	14	13	12	11	21	22	23	24	25	26	27
			47	46	45	44	43	42	41	31	32	33	34	35	36	37
	Schiff index/DH index	Buccal														
	Schiff index/DH index	occlusal														
	Schiff index/DH index	lingual														
	BEWE code	Lingual														
	BEWE code	occlusal														
	BEWE code	Buccal														
	Recession (mm)	Buccal														
		Palatal														
	Probing Depth	Buccal														
		Palatal														
	Bleeding	Buccal														
MANDIBULAR		Palatal														

7.3 Appendix 3 Mean [SD] Median (inter-quartile range) of percentage recession, BEWE and Schiff variables by side of mouth and associated p values

Variable	Side of mouth brushed		p value¶
	Left	Right	
Number of subjects	38	305	
% Recession (Right side of mouth)	12.9 [16.1] 12.5 (0.0-16.7)*	13.6 [16.4] 8.3 (0.0-20.8)**	0.756
% Recession (Left side of mouth)	12.1 [13.9] 8.33 (0.0-16.7)	12.6 [15.3] 8.3 (0.0-18.2)	0.782
% BEWE ≥1 (Right side of mouth)	21.9 [12.3] 20.6 (13.9-30.6)	20.7 [12.8] 19.4 (11.4-28.0)	0.361
% BEWE ≥1 (Left side of mouth)	21.6 [12.2] 23.4 (12.1-30.3)	20.3 [12.6] 18.8 (11.1-28.1)	0.356
% BEWE ≥2 (Right side of mouth)	6.9 [6.4] 5.7 (0.0-11.4)	5.3 [6.6] 2.9 (0.0-8.3)***	0.057
% BEWE ≥2 (Left side of mouth)	6.2 [6.1] 5.6 (0.0-12.1)	4.7 [5.9] 2.8 (0.0-8.3)	0.066
% SCHIFF ≥1 (Right side of mouth)	3.1 [6.2] 0.0 (0.0-2.9)	3.5 [4.8] 2.8 (0.0-5.6)	0.089
% SCHIFF ≥1 (Left side of mouth)	2.8 [5.0] 0.0 (0.0-3.0)	3.4 [4.3] 2.8 (0.0-5.6)	0.135
% SCHIFF ≥2 (Right side of mouth)	1.3 [3.3] 0.0 (0.0-0.0)	1.1 [2.4] 0.0 (0.0-0.0)	0.934
% SCHIFF ≥2 (Left side of mouth)	1.0 [2.2]	1.0 [2.1]	0.941

Variable	Side of mouth brushed		p value¶
	Left	Right	
	0.0 (0.0-0.0)	0.0 (0.0-0.0)	

¶ Mann-Whitney-U test by 'hands'

*/**/*** Wilcoxon matched-pairs, signed-ranks test between left and right sides (p<0.05/<0.01/<0.001)

7.4 Appendix 4 Schiff sextant cumulative score correlations and ANOVA where appropriate (NOTE SUBTEXT*/**)

Variable	Spearman correlation coefficient*	P value for Spearman correlation	P value for ANOVA**
Gender			**0.049**
Location of practice			**0.003**
Location of patient			0.392
Education			0.347
Occupation			**0.002**
Brush frequency	0.025	0.648	
Toothbrush			0.787
Brushing motion			0.796
Brush before breakfast			0.198
Brush after breakfast			**0.002**
Brush after lunch			0.433
Brush after dinner			0.719
delay	-0.091	0.091	
hands			0.812
Sensitivity in previous 12 months			**<0.001**
Indigestion in previous 12 months			**<0.001**
Vomiting in previous 12 months			**0.030**
Eating problem in previous 12 months			**<0.001**
Embarrassed in previous 12 months			**<0.001**
Tense in previous 12 months			**<0.001**
Avoid conversation in previous 12 months			**<0.001**
Sensitivity now			**<0.001**
Sensitivity whilst brushing			**<0.001**
Sensitivity to cold weather			**<0.001**
Sensitivity to touch			**<0.001**
Sensitivity to hot			**<0.001**

Variable	Spearman correlation coefficient*	P value for Spearman correlation	P value for ANOVA**
Sensitivity to sweet			**<0.001**
Sensitivity to cold drink			**<0.001**
Sensitivity due to other			**<0.001**
How long sensitivity occurred			**<0.001**
How important is sensitivity			**<0.001**
Snoring frequency			**<0.001**
Sleep medications			**<0.001**
Smoking			**<0.001**
Chewing gum			**<0.001**
How often have acid foods			**<0.001**
Frequency of acids/day	**0.309**	**<0.001**	
Fresh fruit (how often)			**<0.001**
Fruit juice (how often)			**<0.001**
Isotonic drink (how often)			**<0.001**
Soft drink (how often)			**<0.001**
Cheese (how often)			0.094
Fresh fruit frequency/day			**<0.001**
Juice frequency/day			**<0.001**
Isotonic drink frequency/day			**<0.001**
Soft drink frequency/day			**0.006**
Fresh fruit frequency/day			**0.019**
Last visit to dentist			0.708
Visits to dentist in past 12 months	**0.126**	**0.018**	
Reason for dentist visit			0.031
Height	-0.040	0.453	
Weight	-0.021	0.699	
Sport			**<0.001**
Previous orthodontic appliance			0.400
Currently using a fluoride toothpaste			0.800
Currently using additional fluoride			0.104
Time last acid consumed	**-0.714**	**<0.001**	

Variable	Spearman correlation coefficient*	P value for Spearman correlation	P value for ANOVA**
Percentage of tooth surfaces with recession	0.52	<0.001	
Percentage of tooth surfaces with bleeding	-0.206	<0.001	
BEWE sextant cumulative score			<0.001

*** assuming that variable is either ordinal or quantitative and continuous**

**** assuming that the variable is categorically grouped**

7.5 Appendix 5 BEWE sextant cumulative score correlations and ANOVA where appropriate (NOTE SUBTEXT*/**)

Variable	Spearman correlation coefficient*	P value for Spearman correlation	P value for ANOVA**
Gender			0.086
Location of practice			**0.007**
Location of patient			0.784
Education			0.649
Occupation			0.234
Brush frequency	0.023	0.672	
Toothbrush			0.224
Brushing motion			0.919
Brush before breakfast			0.386
Brush after breakfast			**<0.001**
Brush after lunch			**0.034**
Brush after dinner			0.342
delay	-0.078	0.148	
hands			0.586
Sensitivity in previous 12 months			**<0.001**
Indigestion in previous 12 months			**<0.001**
Vomiting in previous 12 months			0.553
Eating problem in previous 12 months			**<0.001**
Embarrassed in previous 12 months			**<0.001**
Tense in previous 12 months			**<0.001**
Avoid conversation in previous 12 months			**<0.001**
Sensitivity now			**<0.001**
Sensitivity whilst brushing			**<0.001**
Sensitivity to cold weather			**<0.001**
Sensitivity to touch			**<0.001**

Variable	Spearman correlation coefficient*	P value for Spearman correlation	P value for ANOVA**
Sensitivity to hot			**<0.001**
Sensitivity to sweet			**<0.001**
Sensitivity to cold drink			**<0.001**
Sensitivity due to other			**<0.001**
How long sensitivity occurred			**<0.001**
How important is sensitivity			**<0.001**
Snoring frequency			**<0.001**
Sleep medications			**<0.001**
Smoking			**<0.001**
Chewing gum			**0.018**
How often have acid foods			**<0.001**
Frequency of acids/day	**0.290**	**<0.001**	
Fresh fruit (how often)			**<0.001**
Fruit juice (how often)			**<0.001**
Isotonic drink (how often)			**<0.001**
Soft drink (how often)			**<0.001**
Cheese (how often)			0.027
Fresh fruit frequency/day			**<0.001**
Juice frequency/day			**<0.001**
Isotonic drink frequency/day			**<0.001**
Soft drink frequency/day			**<0.001**
Fresh fruit frequency/day			**0.048**
Last visit to dentist			0.834
Visits to dentist in past 12 months	0.078	0.143	
Reason for dentist visit			0.300
Height	-0.018	0.740	
Weight	0.020	0.712	
Sport			**<0.001**
Previous orthodontic appliance			0.908
Currently using a fluoride toothpaste			0.350
Currently using additional fluoride			0.514

Variable	Spearman correlation coefficient*	P value for Spearman correlation	P value for ANOVA**
Time last acid consumed	-0.495	<0.001	
Percentage of tooth surfaces with recession	0.502	<0.001	
Percentage of tooth surfaces with bleeding	0.202	<0.001	
Schiff sextant cumulative score			<0.001

*** assuming that variable is either ordinal or quantitative and continuous**

**** assuming that the variable is categorically grouped**

7.6 Appendix 6 Coefficients, 95% CIs and significance of Schiff sextant cumulative score (dependant variable) to predictor variables

Predictor variable (exposure factor)	Coefficient	95% CI		Significance (p values)
		Lower bound	Upper bound	
BEWE sextant cumulative score	+0.264	+0.184	+0.344	<0.0001
Percentage of tooth surfaces with gingival recession	+0.049	+0.024	+0.074	<0.0001
Sensation to touch	-0.482	-0.877	-0.086	0.017
Sensation to hot	-0.901	-0.408	-1.393	<0.0001

7.7 Appendix 7 Coefficients, 95% CIs and significance of BEWE sextant cumulative score (dependant variable) to predictor variable

Predictor variable (exposure factor)	Coefficient	95% CI		Significance (p values)
		Lower bound	Upper bound	
Tooth sensitivity to hot	+0.684	+1.361	+0.007	0.048
Schiff sextant cumulative score	+0.529	+0.379	+0.679	<0.0001
Brushing after lunch	+0.365	+0.667	+0.064	0.018
Location of practice	+0.208	+0.044	+0.372	0.013
Percentage of tooth surfaces with gingival recession	+0.040	+0.005	+0.074	0.026
If soft drinks are consumed often, how many times a day they are consumed	-0.536	-0.146	-0.925	0.007
Tooth sensitivity to touch	-0.574	-0.044	-1.104	0.034

Bibliography

Abrahamsen TC (2005). The worn dentition--pathognomonic patterns of abrasion and erosion. *Int Dent J* 55(4 Suppl 1)*:*268-276.

Absi EG, Addy M, Adams D (1987). Dentine hypersensitivity. A study of the patency of dentinal tubules in sensitive and non-sensitive cervical dentine. *J Clin Periodontol* 14(5)*:*280-284.

Absi EG, Addy M, Adams D (1989). Dentine hypersensitivity. The development and evaluation of a replica technique to study sensitive and non-sensitive cervical dentine. *J Clin Periodontol* 16(3)*:*190-195.

Absi EG, Addy M, Adams D (1992). Dentine hypersensitivity--the effect of toothbrushing and dietary compounds on dentine in vitro: an SEM study. *J Oral Rehabil* 19(2)*:*101-110.

Absi EG, Addy M, Adams D (1995). Dentine hypersensitivity: uptake of toothpastes onto dentine and effects of brushing, washing and dietary acid--SEM in vitro study. *J Oral Rehabil* 22(3)*:*175-182.

Addy M, Absi EG, Adams D (1987a). Dentine hypersensitivity. The effects *in vitro* of acids and dietary substances on root-planed and burred dentine. *J Clin Periodontol* 14(5)*:*274-279.

Addy M, Griffiths G, Dummer P, Kingdom A, Shaw WC (1987b). The Distribution of Plaque and Gingivitis and the Influence of Toothbrushing Hand in a Group of South-Wales 11-12 Year-Old Children. *J Clin Periodont* 14(10)*:*564-572.

Addy M, Mostafa P, Newcombe R (1987c). Dentine hypersensitivity: a comparison of five toothpastes used during a 6-week treatment period. *Br Dent J* 163(2)*:*45-51.

Addy M, Mostafa P, Newcombe RG (1987d). Dentine hypersensitivity: the distribution of recession, sensitivity and plaque. *J Dent* 15(6)*:*242-248.

Addy M, Mostafa P (1989). Dentine hypersensitivity. II. Effects produced by the uptake in vitro of toothpastes onto dentine. *J Oral Rehabil* 16(1)*:*35-48.

Addy M (1990). Etiology and Clinical Implications of Dentin Hypersensitivity. *Dental Clin of North America* 34(3)*:*503-514.

Addy M, Pearce N (1994). Aetiological, predisposing and environmental factors in dentine hypersensitivity. *Archives of Oral Biology* 39 Suppl(33S-38S.

Addy M (2000). Dentine Hypersensitivity: definition, prevalence, distribution and aetiology. In: Tooth Wear and Sensitivity. M Addy, G Embery, WM Edgar and R Orchardson editors. London: Martin Dunitz, pp. 239-248.

Addy M (2002). Dentine hypersensitivity: new perspectives on an old problem. *Int Dent J* 52(5)*:*367-375.

Addy M, Hughes J, Pickles MJ, Joiner A, Huntington E (2002). Development of a method in situ to study toothpaste abrasion of dentine. Comparison of 2 products. *J Clin Periodontol* 29(10)*:*896-900.

Addy M, Hunter ML (2003). Can tooth brushing damage your health? Effects on oral and dental tissues. *Int Dent J* 53 Suppl 3 (177-186).

Addy M (2005). Tooth brushing, tooth wear and dentine hypersensitivity--are they associated? *Int Dent J* 55(4 Suppl 1)*:*261-267.

Addy M, West NX, Barlow A, Smith S (2007). Dentine hypersensitivity: is there both stimulus and placebo responses in clinical trials? *Int J Dent Hyg* 5(1)*:*53-59.

Ahlquist M, Franzen O, Coffey J, Pashley D (1994). Dental pain evoked by hydrostatic pressures applied to exposed dentin in man: a test of the hydrodynamic theory of dentin sensitivity. *J Endod* 20(3)*:*130-134.

Ahmed TR, Mordan NJ, Gilthorpe MS, Gillam DG (2005). In vitro quantification of changes in human dentine tubule parameters using SEM and digital analysis. *J Oral Rehabil* 32(8)*:*589-597.

Ainamo J, Barmes D, Beagrie G, Cutress T, Martin J, Sardo-Infirri J (1982). Development of the World Health Organization (WHO) community periodontal index of treatment needs (CPITN). *Int Dent J* 32(3)*:*281-291.

Ainamo J, Ainamo A (1985). Partial Indexes as Indicators of the Severity and Prevalence of Periodontal-Disease. *Int Dent J* 35(4)*:*322-326.

Al-Omiri MK, Lamey PJ, Clifford T (2006). Impact of tooth wear on daily living. *Int J Pros* 19(6)*:*601-605.

Albandar JM, Kingman A (1999). Gingival recession, gingival bleeding, and dental calculus in adults 30 years of age and older in the United States, 1988-1994. *J Periodont* 70(1)*:*30-43.

Arends J, Stokroos I, Jongebloed WG, Ruben J (1995). The diameter of dentinal tubules in human coronal dentine after demineralization and air drying. A combined light microscopy and SEM study. *Caries Res* 29(2)*:*118-121.

Asher C, Read MJ (1987). Early enamel erosion in children associated with the excessive consumption of citric acid. *Br Dent J* 162(10)*:*384-387.

Attin T (2006a). Methods for assessment of dental erosion. *Monogr Oral Sci* 20(152-172.

Attin T (2006b). Methods for Assessment of Dental Erosion. In: Dental Erosion From Diagnosis to Therapy. A Lussi editor: Basel: Karger.

Austin RS, Rodriguez JM, Dunne S, Moazzez R, Bartlett DW (2010). The effect of increasing sodium fluoride concentrations on erosion and attrition of enamel and dentine in vitro. *J Dent* 38(10)*:*782-787.

Aw TC, Lepe X, Johnson GH, Mancl L (2002). Characteristics of noncarious cervical lesions: a clinical investigation. *J Am Dent Assoc* 133(6)*:*725-733.

Bamise CT, Olusile AO, Oginni AO, Dosumu OO (2007). The prevalence of dentine hypersensitivity among adult patients attending a Nigerian teaching hospital. *Oral health & prevent dent* 5(1)*:*49-53.

Bamise CT, Olusile AO, Oginni AO (2008). An analysis of the etiological and predisposing factors related to dentin hypersensitivity. *J Contemp Dent Pract* 9(5)*:*52-59.

Banfield N, Addy M (2004). Dentine hypersensitivity: development and evaluation of a model in situ to study tubule patency. *J Clin Periodontol* 31(5)*:*325-335.

Bardsley PF, Taylor S, Milosevic A (2004). Epidemiological studies of tooth wear and dental erosion in 14-year-old children in North West England. Part 1: The relationship with water fluoridation and social deprivation. *Br Dent J* 197(7)*:*413-416.

Bardsley PF (2008). The evolution of tooth wear indices. *Clin Oral Investig* 12 Suppl 1(S15-19.

Barlow AP, Parkinson C, Butler A, Mason SC, Addy M, West N (2007). In situ investigation of dentine tubule occlusion. IADR/AADR/CADR 85th General Session and Exhibition. New Orleans.

Bartlett D (2005a). The implication of laboratory research on tooth wear and erosion. *Oral diseases* 11(1)*:*3-6.

Bartlett D, Ganss C, Lussi A (2008). Basic Erosive Wear Examination (BEWE): a new scoring system for scientific and clinical needs. *Clin Oral Investig* 12 Suppl 1(S65-68.

Bartlett D, Harding M, Sherriff M, Shirodaria S, Whelton H (2011a). A new index to measure tooth wear--methodolgy and practical advice. *Community dental health* 28(2)*:*182-187.

Bartlett DW, Blunt L, Smith BG (1997). Measurement of tooth wear in patients with palatal erosion. *Br Dent J* 182(5)*:*179-184.

Bartlett DW, Coward PY, Nikkah C, Wilson RF (1998). The prevalence of tooth wear in a cluster sample of adolescent schoolchildren and its relationship with potential explanatory factors. *Br Dent J* 184(3)*:*125-129.

Bartlett DW, Ide M (1999). Dealing with sensitive teeth. *Prim Dent Care* 6(1)*:*25-27.

Bartlett DW, Smith BGN (2000). Definition, classification and clinical assessment of attrition, erosion and abrasion of enamel and dentine. In: Tooth wear and sensitivity - clinical advances in restorative dentistry. M Addy, G Embery, WM Edgar and R Orchardson editors. London: Martin Dunitz.

Bartlett DW (2003). Retrospective long term monitoring of tooth wear using study models. *Br Dent J* 194(4)*:*211-213; discussion 204.

Bartlett DW (2005b). The role of erosion in tooth wear: aetiology, prevention and management. *Int Dent J* 55(4 Suppl 1)*:*277-284.

Bartlett DW, Palmer I, Shah P (2005). An audit of study casts used to monitor tooth wear in general practice. *Br Dent J* 199(3)*:*143-145.

Bartlett DW, Shah P (2006). A critical review of non-carious cervical (wear) lesions and the role of abfraction, erosion, and abrasion. *J Dent Res* 85(4)*:*306-312.

Bartlett DW, Fares J, Shirodaria S, Chiu K, Ahmad N, Sherriff M (2011b). The association of tooth wear, diet and dietary habits in adults aged 18-30 years old. *J Dent* 39(12)*:*811-816.

Bartold PM (2006). Dentinal hypersensitivity: a review. *Aust Dent J* 51(3)*:*212-218; quiz 276.

Bassani DG, da Silva CM, Oppermann RV (2006). Validity of the "Community Periodontal Index of Treatment Needs" (CPITN) for population periodontitis screening. *Cadernos de saude publica / Ministerio da Saude, Fundacao Oswaldo Cruz, Escola Nacional de Saude Publica* 22(2)*:*277-283.

Battjes KP (2004) Microscopy and x-ray elemental spectroscopy in failure analysis: case studies. *ANTEC:*3034-3038.

Bekes K, John MT, Schaller HG, Hirsch C (2009). Oral health-related quality of life in patients seeking care for dentin hypersensitivity. *J Oral Rehabil* 36(1)*:*45-51.

Bender H (1999). Focused ion beam specimen preparation for transmission electron microscopy studies of ULSI devices. *Inst Phys Conf Ser* 164)*:*593-602.

Berry DC, Poole DF (1974). Masticatory function and oral rehabilitation. *J Oral Rehabil* 1(2)*:*191-205.

Betke H, Schick U, Buchalla W, Hellwig E, Attin T (2003). [Influence of the buffer capacity of amine fluoride-containing toothpastes and gels in enamel erosion]. *Schweiz Monatsschr Zahnmed* 113(11)*:*1158-1164.

Bevenius J, L'Estrange P, Karlsson S, Carlsson GE (1993). Idiopathic cervical lesions: in vivo investigation by oral microendoscopy and scanning electron microscopy. A pilot study. *J Oral Rehabil* 20(1)*:*1-9.

Boiko OV, Baker SR, Gibson BJ, Locker D, Sufi F, Barlow AP *et al.* (2010). Construction and validation of the quality of life measure for dentine hypersensitivity (DHEQ). *J Clin Periodontol* 37(11)*:*973-980.

Boyde A, Xiao GQ, Corle T, Watson TF, Kino GS (1990). An Evaluation of Unilateral Tsm for Biological Applications. *Scanning* 12(5)*:*273-279.

Brannstrom M (1963). A hydrodynamic mechanism in the transmission of pain producing stimuli through the dentine. In: Sensory Mechanisms in Dentine. D Anderson editor. Oxford: Pergamon press, pp. 73-79.

Brannstrom M (1965). The surface of sensitive dentine. An experimental study using replication. *Odontologisk revy* 16(4)*:*293-299.

Brannstrom M (1966). Sensitivity of dentine. *Oral Surg Oral Med Oral Pathol* 21(4)*:*517-526.

Brannstrom M, Johnson G (1978). The sensory mechanism in human dentin as revealed by evaporation and mechanical removal of dentin. *J Dent Res* 57(1)*:*49-53.

Broca P (1879). Instructions rélatives a l'étude anthropologique du systéme dentaire. *Bull Soc Anthrop Paris* 2(3)*:*128-163.

BSDA (2011). The 2011 Soft Drinks Report. London.

Burwell A, Jennings D, Muscle D, Greenspan DC (2010). NovaMin and dentin hypersensitivity--in vitro evidence of efficacy. *J Clin Dent* 21(3)*:*66-71.

Canadian, Advisory, board, on, dentine, hypersensitivity (2003). Consensus-based recommendations for the diagnosis and management of dentin hypersensitivity. *J Can Dent Assoc* 69(4)*:*221-226.

Chabanski MB, Dummer PM, Hunter MI, A. K, Shaw WC (1990). The effect of tooth brushing frequency, tooth brushing hand, sex and social class on the incidence of plaque, gingivitis and pocketing in adolescents: a longitudinal cohort study. *Community dental health* 7(237-247.

Chabanski MB, Gillam DG, Bulman JS, Newman HN (1996). Prevalence of cervical dentine sensitivity in a population of patients referred to a specialist Periodontology Department. *J Clin Periodontol* 23(11)*:*989-992.

Chabanski MB, Gillam DG (1997). Aetiology, prevalence and clinical features of cervical dentine sensitivity. *J Oral Rehabil* 24(1)*:*15-19.

Chabanski MB, Gillam DG, Bulman JS, Newman HN (1997). Clinical evaluation of cervical dentine sensitivity in a population of patients referred to a specialist periodontology department: a pilot study. *J Oral Rehabil* 24(9)*:*666-672.

Chadwick B, Penry L (2004). Children's dental health in the United Kingdom 2003: Non carious dental conditions. London: The Stationary Office.

Chadwick RG, Mitchell HL, Cameron I, Hunter B, Tulley M (1997). Development of a novel system for assessing tooth and restoration wear. *J Dent* 25(1)*:*41-47.

Chadwick RG (2006). Dental Erosion London: Quintessence Publishing Co. Ltd.

Choi S, Park KH, Cheong Y, Moon SW, Park YG, Park HK (2012). Potential effects of tooth-brushing on human dentin wear following exposure to acidic soft drinks. *J Microsc* 247(2)*:*176-185.

Ciocca L, Gallina I, Navacchia E, Baldissara P, Scotti R (2007). A new method for quantitative analysis of dentinal tubules. *Computers in biology and medicine* 37(3)*:*277-286.

Claydon NC, Addy M, MacDonald EL, West NX, Maggio B, Barlow A *et al.* (2009). Development of an in situ methodology for the clinical evaluation of dentine hypersensitivity occlusion ingredients. *J Clin Dent* 20(5)*:*158-166.

Curro FA, Friedman M, Leight RS (2000). Design and conduct of clinical trials on dentine hypersensitivity. In: Tooth Wear and Sensitivity. M Addy, G Emberry, G Edgar and R Orchardson editors. London: Martin Duntiz, pp. 299-314.

Dababneh RH, Khouri AT, Addy M (1999). Dentine hypersensitivity - an enigma? A review of terminology, mechanisms, aetiology and management. *Br Dent J* 187(11)*:*606-611; discussion 603.

Dahl BL, Oilo G, Andersen A, Bruaset O (1989). The suitability of a new index for the evaluation of dental wear. *Acta odontologica Scandinavica* 47(4)*:*205-210.

Davies M, Paice EM, Jones SB, Leary S, Curtis AR, West NX (2011). Efficacy of desensitizing dentifrices to occlude dentinal tubules. *Eur J Oral Sci* 119(6)*:*497-503.

Davies R, Scully C, Preston AJ (2010). Dentifrices--an update. *Medicina oral, patologia oral y cirugia bucal* 15(6)*:*e976-982.

Deery C, Wagner ML, Longbottom C, Simon R, Nugent ZJ (2000). The prevalence of dental erosion in a United States and a United Kingdom sample of adolescents. *Pediatric dentistry* 22(6)*:*505-510.

Diamanti-Kipioti A, Papapanou PN, Moraitaki-Tsami A, Lindhe J, Mitsis F (1993). Comparative estimation of periodontal conditions by means of different index systems. *J Clin Periodontol* 20(9)*:*656-661.

Dictitionary OE "disease, n". In: O Online editor: Oxford University Press.

Dictitionary OE (2012). "model, n and adj.". OED Online: Oxford University Press.

Dixon B, Sharif MO, Ahmed F, Smith, AB, Seymour D, Brunton PA (2012) Evaluation of the basic erosive wear examination (BEWE) for use in general dental practice. *Br Dent J* 213

DoH (2009). Delivering Better Oral Health: An evidence-based toolkit for prevention. 2nd Ed. Department of Health/British Association for the Study of Community Dentistry.

Donachie MA, Walls AWG (1995). Assessment of Tooth Wear in an Aging Population. *J of Dent* 23(3)*:*157-164.

Donachie MA, Walls AWG (1996). The tooth wear index: A flawed epidemiological tool in an ageing population group. *Comm dent and oral epidem* 24(2)*:*152-158.

Dowell P, Addy M (1983). Dentine hypersensitivity--a review. Aetiology, symptoms and theories of pain production. *J Clin Periodontol* 10(4)*:*341-350.

Dowell P, Addy M, Dummer P (1985). Dentine hypersensitivity: aetiology, differential diagnosis and management. *Br Dent J* 158(3)*:*92-96.

Draaijer A, Houpt PM (1988). A Standard Video-Rate Confocal Laser-Scanning Reflection and Fluorescence Microscope. *Scanning* 10(4)*:*139-145.

Drisko CH (2002). Dentine hypersensitivity - dental hygiene and periodontal considerations. *Int Dent J* 52(5)*:*385-393.

Dugmore CR, Rock WP (2003). The progression of tooth erosion in a cohort of adolescents of mixed ethnicity. *International journal of paediatric dentistry / the British Paedodontic Society [and] the International Association of Dentistry for Children* 13(5)*:*295-303.

Dugmore CR, Rock WP (2004). The prevalence of tooth erosion in 12-year-old children. *Br Dent J* 196(5)*:*279-282; discussion 273.

Earl JS, Leary RK, Perrin JS, Brydson R, Harrington JP, Markowitz K *et al.* (2010a). Characterization of dentine structure in three dimensions using FIB-SEM. *J Microsc-Oxford* 240(1)*:*1-5.

Earl JS, Ward MB, Langford RM (2010b). Investigation of dentinal tubule occlusion using FIB-SEM milling and EDX. *J Clin Dent* 21(2)*:*37-41.

Eccles JD (1978). The treatment of dental erosion. *J Dent* 6(3)*:*217-221.

Eccles JD (1979). Dental erosion of nonindustrial origin. A clinical survey and classification. *J Prosthet Dent* 42(6)*:*649-653.

Egerton RF (2008). Physical principles of electron microscopy: an introduction to TEM, SEM and AEM. 2nd ed.

El Aidi H, Bronkhorst EM, Truin GJ (2008). A longitudinal study of tooth erosion in adolescents. *J Dent Res* 87(8)*:*731-735.

Elias A, Sheiham A (1998). The relationship between satisfaction with mouth and number and position of teeth. *J of Oral Rehab* 25(9)*:*649-661.

Fares J, Shirodaria S, Chiu K, Ahmad N, Sherriff M, Bartlett D (2009). A new index of tooth wear. Reproducibility and application to a sample of 18- to 30-year-old university students. *Caries Res* 43(2)*:*119-125.

Fischer C, Fischer RG, Wennberg A (1992). Prevalence and distribution of cervical dentine hypersensitivity in a population in Rio de Janeiro, Brazil. *J Dent* 20(5)*:*272-276.

Fleiss JL, Cohen J (1973). Equivalence of Weighted Kappa and Intraclass Correlation Coefficient as Measures of Reliability. *Educ Psychol Meas* 33(3)*:*613-619.

Flynn J, Galloway R, Orchardson R (1985). The incidence of 'hypersensitive' teeth in the West of Scotland. *J Dent* 13(3)*:*230-236.

Fosse G, Saele PK, Eide R (1992). Numerical density and distributional pattern of dentin tubules. *Acta odontologica Scandinavica* 50(4)*:*201-210.

Fu Y, Li X, Que K, Wang M, Hu D, Mateo LR *et al.* (2010). Instant dentin hypersensitivity relief of a new desensitizing dentifrice containing 8.0% arginine, a high cleaning calcium carbonate system and 1450ppm fluoride: a 3-day clinical study in Chengdu, China. *American J of Dent* 23 Spec No A(20A-27A.

Ganss C, Klimek J, Giese K (2001). Dental erosion in children and adolescents--a cross-sectional and longitudinal investigation using study models. *Comm dent and oral epidemiology* 29(4)*:*264-271.

Ganss C, Klimek J, Lussi A (2006). Accuracy and consistency of the visual diagnosis of exposed dentine on worn occlusal/incisal surfaces. *Caries Res* 40(3)*:*208-212.

Ganss C, Lussi A (2006). Diagnosis of erosive tooth wear. *Monogr Oral Sci* 20(32-43.

Ganss C, Hardt M, Blazek D, Klimek J, Schlueter N (2009). Effects of toothbrushing force on the mineral content and demineralized organic matrix of eroded dentine. *Eur J Oral Sci* 117(3)*:*255-260.

Garberoglio R, Brannstrom M (1976). Scanning electron microscopic investigation of human dentinal tubules. *Arch Oral Biol* 21(6)*:*355-362.

Garcia-Godoy F (2009). Dentin hypersensitivity: beneficial effects of an arginine-calcium carbonate desensitizing paste. *American J of Dent* 22 Spec No A(2A.

Gibson B, Boiko O, Baker S, Robinson PG, Barlow A, Player T *et al.* (2010). The everyday impact of dentine sensitivity: personal and functional aspects. *Social Science and Dentistry* 1(11-20).

Gillam D.G., Orchardson R, Narhi MN, V. K-N (2000). Present and future methods for the evaluation of pain associated with dentine hypersensitivity. In: Tooth Wear and Sensitivity. M Addy, E G. and E W.M. editors. London: Martin Dunitz, pp. 283-298.

Gillam DG, Seo HS, Bulman JS, Newman HN (1999). Perceptions of dentine hypersensitivity in a general practice population. *J Oral Rehabil* 26(9)*:*710-714.

Gillam DG, Orchardson R, Narhi MVO, Kontturi-Narhi V (2000). Present and future methods for the evaluation of pain associated with dentine hypersensitivity. In: Tooth Wear and Sensitivity. M Addy, G Embery, G Edgar, W Edgar and R Orchardson editors. London, UK: Martin Dunitz.

Gillam DG, Tang JY, Mordan NJ, Newman HN (2002). The effects of a novel Bioglass dentifrice on dentine sensitivity: a scanning electron microscopy investigation. *J Oral Rehabil* 29(4)*:*305-313.

Goodhew PJ, Humphreys FJ, Beanland R (2000). Electron microscopy and analysis.

Graf H, Galasse R (1977). Morbidity, Prevalence and Intraoral Distribution of Hypersensitive Teeth. *J of dent res* 56(A162-A162.

Gregg T, Mace S, West NX, Addy M (2004). A study in vitro of the abrasive effect of the tongue on enamel and dentine softened by acid erosion. *Caries Res* 38(6)*:*557-560.

Gregoire G, Joniot S, Guignes P, Millas A (2003). Dentin permeability: self-etching and one-bottle dentin bonding systems. *J Prosthet Dent* 90(1)*:*42-49.

Grenby TH, Phillips A, Desai T, Mistry M (1989). Laboratory studies of the dental properties of soft drinks. *The British journal of nutrition* 62(2)*:*451-464.

Grenby TH (1996). Methods of assessing erosion and erosive potential. *Eur J Oral Sci* 104(2 (Pt 2))*:*207-214.

Grippo JO (1991b). Tooth flexure. *J Am Dent Assoc* 122(7)*:*13.

Grippo JO, Simring M, Schreiner S (2004). Attrition, abrasion, corrosion and abfraction revisited: a new perspective on tooth surface lesions. *J Am Dent Assoc* 135(8)*:*1109-1118; quiz 1163-1105.

Gysi A (1900). An attempt to explain the sensitiveness of dentin. *Brit J of Dent Sci* 43(865-868.

Hall RC, Embery G, Shellis RP (2000). Biological and structural features of enamel and dentine hypersensitivity. In: Tooth Wear and Sensitivity. M Addy, G Embery, WM Edgar and R Orchardson editors. London: Martin Dunitz.

Harding MA, Whelton H, O'Mullane DM, Cronin M (2003). Dental erosion in 5-year-old Irish school children and associated factors: a pilot study. *Community dental health* 20(3)*:*165-170.

Harding MA, Whelton HP, Shirodaria SC, O'Mullane DM, Cronin MS (2010). Is tooth wear in the primary dentition predictive of tooth wear in the permanent dentition? Report from a longitudinal study. *Community dental health* 27(1)*:*41-45.

Hefferen JJ (1976). A laboratory method for assessment of dentifrice abrasivity. *J of dent res* 55(563-573.

Hodge HC, Gavett E, Thomas I (1946). The adsorption of strontium at forty degrees by enamel, dentin, bone, and hydroxyapatite as shown by the radioactive isotope. *J of biol chem* 163(1-6.

Holbrook WP, Ganss C (2008). Is diagnosing exposed dentine a suitable tool for grading erosive loss? *Clin Oral Invest* 12(S33-S39.

Holland GR, Narhi MN, Addy M, Gangarosa L, Orchardson R (1997). Guidelines for the design and conduct of clinical trials on dentine hypersensitivity. *J Clin Periodontol* 24(11)*:*808-813.

Hooper S, West NX, Pickles MJ, Joiner A, Newcombe RG, Addy M (2003). Investigation of erosion and abrasion on enamel and dentine: a model in situ using toothpastes of different abrasivity. *J Clin Periodontol* 30(9)*:*802-808.

Hooper SM, Hughes JA, Newcombe RG, Addy M, West NX (2005). A methodology for testing the erosive potential of sports drinks. *J of Dent* 33(4)*:*343-348.

Hughes JA, West NX, Parker DM, Newcombe RG, Addy M (1999). Development and evaluation of a low erosive blackcurrant juice drink. 3. Final drink and concentrate, formulae comparisons in situ and overview of the concept. *J Dent* 27(5)*:*345-350.

Hughes N, Mason S, Jeffery P, Welton H, Tobin M, O'Shea C *et al.* (2010). A comparative clinical study investigating the efficacy of a test dentifrice containing 8% strontium acetate and 1040ppm sodium fluoride versus a marketed control dentifrice containing 8% arginine, calcium carbonate, and 1450ppm sodium monofluorophosphate in reducing dentinal hypersensitivity. *J of Clin Dent* 21(2)*:*49-55.

Hugoson A, Bergendal T, Ekfeldt A, Helkimo M (1988). Prevalence and severity of incisal and occlusal tooth wear in an adult Swedish population. *Acta odontologica Scandinavica* 46(5)*:*255-265.

Hunter ML, West NX, Hughes JA, Newcombe RG, Addy M (2000). Erosion of deciduous and permanent dental hard tissue in the oral environment. *J Dent* 28(4)*:*257-263.

Hunter ML, Addy M, Pickles MJ, Joiner A (2002). The role of toothpastes and toothbrushes in the aetiology of tooth wear. *Int Dent J* 52(5)*:*399-405.

Ide M, Wilson RF, Ashley FP (2001). The reproducibility of methods of assessment for cervical dentine hypersensitivity. *J Clin Periodontol* 28(1)*:*16-22.

International.Standards.Organisation.ISO.11609 (1995). Dentistry - toothpastes-requirements, test methods and marking. Geneva: International Organisation for Standardisation.

Ishikawa S (1969). [A clinico-histological study on the hypersensitivity of dentin]. *Kokubyo Gakkai zasshi The Journal of the Stomatological Society, Japan* 36(4)*:*278-298.

Johnson RH, Zulqar-Nain BJ, Koval JJ (1982). The effectiveness of an electro-ionizing toothbrush in the control of dentinal hypersensitivity. *J Periodontol* 53(6)*:*353-359.

Kanapka JA (1990). Over-the-counter dentifrices in the treatment of tooth hypersensitivity. Review of clinical studies. *Dent Clin of North Am* 34(3)*:*545-560.

Kelly M, Steele J, Nuttall N, Bradnock G, Morris J, Nunn J (1998). Adult Dental Health Survey.

Kerdvongbundit V, Thiradilok S, Vongsavan N, Matthews B (2004). The use of the replica technique to record fluid emerging from exposed dentine. *Archives of Oral Biology* 49(8)*:*613-619.

Khan F, Young WG, Shahabi S, Daley TJ (1999). Dental cervical lesions associated with occlusal erosion and attrition. *Aust Dent J* 44(3)*:*176-186.

Kidd EAM (2005). Prevention of caries by plaque control. In: Essentials of Dental Caries. EAM Kidd editor. London: Oxford University Press, pp. 77-80.

Kleinberg I (2002). SensiStat. A new saliva-based composition for simple and effective treatment of dentinal sensitivity pain. *Dent Today* 21(12)*:*42-47.

Klineberg I, H.W. K, Confessore F (1990). Methods in measuring tooth hypersensitivity. *34:*515-529.

Knezevic A, Nyamaa I, Tarle Z, Kunzelmann KH (2010). In vitro assessment of human dentin wear resulting from toothbrushing. *J Calif Dent Assoc* 38(2)*:*109-113.

Krauser JT (1986a). Hypersensitive teeth. Part I: Etiology. *J Prosthet Dent* 56(2)*:*153-156.

Krauser JT (1986b). Hypersensitive teeth. Part II: Treatment. *J Prosthet Dent* 56(3)*:*307-311.

Kubinek R, Zapletalova Z, Vujtek M, Novotny R, Kolarova H, Chmelickova H *et al.* (2007). Sealing of open dentinal tubules by laser irradiation: AFM and SEM observations of dentine surfaces. *J Mol Recognit* 20(6)*:*476-482.

Langford RM (2006). Focused ion beams techniques for nanomaterials characterization. *Microsc Res Techniq* 69(7)*:*538-549.

Larsen IB, Westergaard J, Stoltze K, Larsen AI, Gyntelberg F, Holmstrup P (2000). A clinical index for evaluating and monitoring dental erosion. *Comm dent and oral epidem* 28(3)*:*211-217.

Lavender SA, Petrou I, Heu R, Stranick MA, Cummins D, Kilpatrick-Liverman L *et al.* (2010). Mode of action studies on a new desensitizing dentifrice containing 8.0% arginine, a high cleaning calcium carbonate system and 1450ppm fluoride. *Am J of Dent* 23 Spec No A(14A-19A.

Lee SY, Kwon HK, Kim BI (2008). Effect of dentinal tubule occlusion by dentifrice containing nano-carbonate apatite. *J Oral Rehabil* 35(11)*:*847-853.

Lee WC, Eakle WS (1984). Possible role of tensile stress in the etiology of cervical erosive lesions of teeth. *J Prosthet Dent* 52(3)*:*374-380.

Ling TY, Gillam DG (1996). The effectiveness of desensitizing agents for the treatment of cervical dentine sensitivity (CDS)--a review. *J West Soc Periodontol Periodontal Abstr* 44(1)*:*5-12.

Ling TY, Gillam DG, Barber PM, Mordan NJ, Critchell J (1997). An investigation of potential desensitizing agents in the dentine disc model: a scanning electron microscopy study. *J Oral Rehabil* 24(3)*:*191-203.

Linkosalo E, Markkanen H (1985). Dental erosions in relation to lactovegetarian diet. *Scand J of dent res* 93(5)*:*436-441.

Linsuwanont P, Palamara JE, Messer HH (2008). Thermal transfer in extracted incisors during thermal pulp sensitivity testing. *Int endo journal* 41(3)*:*204-210.

Liu HC, Lan WH, Hsieh CC (1998). Prevalence and distribution of cervical dentin hypersensitivity in a population in Taipei, Taiwan. *J Endod* 24(1)*:*45-47.

Liu X, Barnes V, DeVizio W, Yang H, Malmstrom H, Ren Y (2011). Effects of dentin tubule occlusion by dentifrice containing a PVM/MA bioadhesive copolymer in a silica base. *J Dent* 39(4)*:*293-301.

Lussi A, Schaffner M, Hotz P, Suter P (1991). Dental erosion in a population of Swiss adults. *Comm dentistry and oral epidemiology* 19(5)*:*286-290.

Lussi A, Schaffner M (2000). Progression of and risk factors for dental erosion and wedge-shaped defects over a 6-year period. *Caries Res* 34(2)*:*182-187.

Lussi A (2006). Dental Erosion From Diagnosis to Therapy: Karger.

Lussi A, Hellwig E, Zero D, Jaeggi T (2006). Erosive tooth wear: diagnosis, risk factors and prevention. *Am J Dent* 19(6)*:*319-325.

Macdonald E, North A, Maggio B, Sufi F, Mason S, Moore C *et al.* (2010). Clinical study investigating abrasive effects of three toothpastes and water in an in situ model. *J Dent* 38(6)*:*509-516.

Madhu PS, Setty S, Ravindra, S. (2006). Dentinal hypersensitivity?-Can this agent be the solution? *J of dent res* 17(178-184.

Mahoney E, Beattie J, Swain M, Kilpatrick N (2003). Preliminary in vitro assessment of erosive potential using the ultra-micro-indentation system. *Caries Res* 37(3)*:*218-224.

Markowitz K, Pashley DH (2008). Discovering new treatments for sensitive teeth: the long path from biology to therapy. *J Oral Rehabil* 35(4)*:*300-315.

Marshall GW, Marshall SJ, Kinney JH, Balooch M (1997). The dentin substrate: structure and properties related to bonding. *Journal of Dentistry* 25(6)*:*441-458.

Mason S, Hughes N, Sufi F, Bannon L, Maggio B, North M *et al.* (2010). A comparative clinical study investigating the efficacy of a dentifrice containing 8% strontium acetate and 1040ppm fluoride in a silica base and a control dentifrice containing 1450ppm fluoride in a silica base to provide immediate relief of dentin hypersensitivity. *J Clin Dent* 21(2)*:*42-48.

McCracken GI, Janssen J, Swan M, Steen N, de Jager M, Heasman PA (2003). Effect of brushing force and time on plaque removal using a powered toothbrush. *J Clin Periodontol* 30(5)*:*409-413.

McGrath PA (1986). The measurement of human pain. *Endodontics & dental traumatology* 2(4)*:*124-129.

Meurman JH, Rytomaa I, Kari K, Laakso T, Murtomaa H (1987). Salivary pH and glucose after consuming various beverages, including sugar-containing drinks. *Caries Res* 21(4)*:*353-359.

Meurman JH, Drysdale T, Frank RM (1991). Experimental erosion of dentin. *Scand J of Dent Res* 99(6)*:*457-462.

Meurman JH, Frank RM (1991). Progression and surface ultrastructure of in vitro caused erosive lesions in human and bovine enamel. *Caries Res* 25(2)*:*81-87.

Meurman JH, tenCate JM (1996). Pathogenesis and modifying factors of dental erosion. *Euro J of Oral Sci* 104(2)*:*199-206.

Millward A, Shaw L, Smith AJ, Rippin JW, Harrington E (1994). The distribution and severity of tooth wear and the relationship between erosion and dietary constituents in a group of children. *International journal of paediatric dentistry / the British Paedodontic Society [and] the International Association of Dentistry for Children* 4(3)*:*151-157.

Milosevic A, Young PJ, Lennon MA (1994). The prevalence of tooth wear in 14-year-old school children in Liverpool. *Community dental health* 11(2)*:*83-86.

Milosevic A, Lennon MA, Fear SC (1997). Risk factors associated with tooth wear in teenagers: a case control study. *Community dental health* 14(3)*:*143-147.

Mjor IA, Nordahl I (1996). The density and branching of dentinal tubules in human teeth. *Arch Oral Biol* 41(5)*:*401-412.

Moss SJ (1998). Dental erosion. *Int Dent J* 48(6)*:*529-539.

Mulic A, Tveit AB, Wang NJ, Hove LH, Espelid I, Skaare AB (2010). Reliability of two clinical scoring systems for dental erosive wear. *Caries Res* 44(3)*:*294-299.

Murayama Y, Omata S (2004). Fabrication of micro tactile sensor for the measurement of micro-scale local elasticity. *Sensor Actuat a-Phys* 109(3)*:*202-207.

Nunn J, Morris J, Pine C, Pitts NB, Bradnock G, Steele J (2000). The condition of teeth in the UK in 1998 and implications for the future. *Br Dent J* 189(12)*:*639-644.

Nunn JH (1996). Prevalence of dental erosion and the implications for oral health. *Eur J Oral Sci* 104(2 (Pt 2)):156-161.

Nunn JH, Gordon PH, Morris AJ, Pine CM, Walker A (2003). Dental erosion -- changing prevalence? A review of British National childrens' surveys. *International journal of paediatric dentistry / the British Paedodontic Society [and] the International Association of Dentistry for Children* 13(2):98-105.

O'Brien M (1993). Children's dental health in the United Kingdom 1993. London.

O'Brien M (1994). Children's dental health in the United Kingdom, 1993: Non carious dental conditions. London: The Stationary Office.

Oilo G, Dahl BL, Hatle G, Gad AL (1987). An Index for Evaluating Wear of Teeth. *Acta odontologica Scandinavica* 45(5):361-&.

Olley RC, Gallagher JE (2010). Tobacco usage and control: information and advice for primary dental care practitioners. *Dent Update* 37(1):40-42, 45-46, 49-50 passim.

Olsson S, Oilo G, Adamczak E (1993). The structure of dentin surfaces exposed for bond strength measurements. *Scandinavian journal of dental research* 101(3):180-184.

Orchardson R, Collins WJ (1987a). Thresholds of hypersensitive teeth to 2 forms of controlled stimulation. *J Clin Periodontol* 14(2):68-73.

Orchardson R, Collins WJ (1987b). Clinical features of hypersensitive teeth. *Br Dent J* 162(7):253-256.

Orchardson R, Gangarosa LP, Sr., Holland GR, Pashley DH (1994). Towards a standard code of practice for evaluating the effectiveness of treatments for hypersensitive dentine. *Arch Oral Biol* 39 Suppl(121S-124S.

Parkinson CR, Butler A, Willson RJ (2010). Development of an acid challenge-based in vitro dentin disc occlusion model. *J of Clin Dent* 21(2):31-36.

Parkinson CR, Willson RJ (2011a). A comparative in vitro study investigating the occlusion and mineralization properties of commercial toothpastes in a four-day dentin disc model. *J Clin Dent* 22(3):74-81.

Parkinson CR, Willson RJ (2011b). An in vitro investigation of two currently marketed dentin tubule occlusion dentifrices. *J Clin Dent* 22(1):6-10.

Pashley D (1990a). Dentin permeability: Theory and practice: Boca Raton, FL: CRC Press.

Pashley DH, Michelich V, Kehl T (1981). Dentin permeability: effects of smear layer removal. *J Prosthet Dent* 46(5):531-537.

Pashley DH (1984). Smear layer: physiological considerations. *Oper Dent Suppl* 3(13-29.

Pashley DH (1986). Dentin permeability, dentin sensitivity, and treatment through tubule occlusion. *J Endod* 12(10):465-474.

Pashley DH, Derkson GD, Tao L, Derkson M, Kalathoor S (1988). The effects of a multi-step dentin bonding system on dentin permeability. *Dent Mater* 4(2):60-63.

Pashley DH (1989). Dentin: a dynamic substrate--a review. *Scanning microscopy* 3(1):161-174; discussion 174-166.

Pashley DH (1990b). Dentine permeability: theory and practice. In: Experimental Endodontics. S L. editor.

Pashley DH (1990c). Mechanisms of dentin sensitivity. *Dent Clin North Am* 34(3)*:*449-473.

Pashley DH (1994). Dentine permeability and its role in the pathobiology of dentine sensitivity. *Arch Oral Biol* 39 Suppl(73S-80S.

Pashley DH, Pashley EL, Carvalho RM, Tay FR (2002). The effects of dentin permeability on restorative dentistry. *Dent Clin North Am* 46(2)*:*211-245, v-vi.

Pawley JB (1995). Handbook of Biological Confocal Microscopy 2nd Edition New York.

Penniston KL, Nakada SY, Holmes RP, Assimos DG (2008). Quantitative assessment of citric acid in lemon juice, lime juice, and commercially-available fruit juice products. *Journal of endourology / Endourological Society* 22(3)*:*567-570.

Perdigao J, Lambrechts P, Van Meerbeek B, Vanherle G, Lopes AL (1995). Field emission SEM comparison of four postfixation drying techniques for human dentin. *J of biomed mat res* 29(9)*:*1111-1120.

Pereira JC, Segala AD, Gillam DG (2005). Effect of desensitizing agents on the hydraulic conductance of human dentin subjected to different surface pre-treatments--an in vitro study. *Dent Mater* 21(2)*:*129-138.

Petrou I, Heu R, Stranick M, Lavender S, Zaidel L, Cummins D *et al.* (2009). A breakthrough therapy for dentin hypersensitivity: how dental products containing 8% arginine and calcium carbonate work to deliver effective relief of sensitive teeth. *J Clin Dent* 20(1)*:*23-31.

Pickles MJ, Joiner A, Weader E, Cooper YL, Cox TF (2005). Abrasion of human enamel and dentine caused by toothpastes of differing abrasivity determined using an in situ wear model. *Int Dent J* 55(3)*:*188-193.

Porritt, JM, Robinson PG, Gibson BJ, Baker S (2012). Illness Beliefs, Coping and Impacts Associated with Dentine Hypersensitivity. IADR/AADR/CADR 90th General Session and Exhibition. Foz du Iguacu.

Porter AE, Nalla RK, Minor A, Jinschek JR, Kisielowski C, Radmilovic V *et al.* (2005). A transmission electron microscopy study of mineralization in age-induced transparent dentin. *Biomaterials* 26(36)*:*7650-7660.

Poulsen S, Errboe M, Lescay Mevil Y, Glenny AM (2006). Potassium containing toothpastes for dentine hypersensitivity. *Cochrane Database Syst Rev* 3)*:*CD001476.

Poynter ME, Wright PS (1990). Tooth wear and some factors influencing its severity. *Rest dent* 6(4)*:*8-11.

Pradeep AR, Sharma A (2010). Comparison of clinical efficacy of a dentifrice containing calcium sodium phosphosilicate to a dentifrice containing potassium nitrate and to a placebo on dentinal hypersensitivity: a randomized clinical trial. *J Periodontol* 81(8)*:*1167-1173.

Prati C, Erickson R, Tao L, Simpson M, Pashley DH (1991). Measurement of dentin permeability and wetness by use of the Periotron device. *Dent Mater* 7(4)*:*268-273.

Que K, Fu Y, Lin L, Hu D, Zhang YP, Panagakos FS *et al.* (2010a). Dentin hypersensitivity reduction of a new toothpaste containing 8.0% arginine and 1450ppm fluoride: an 8-week clinical study on Chinese adults. *Am J of Dent* 23 Spec No A(28A-35A.

Que K, Ruan J, Fan X, Liang X, Hu D (2010b). A multi-centre and cross-sectional study of dentine hypersensitivity in China. *J Clin Periodontol* 37(7)*:*631-637.

Radentz WH, Barnes GP, Cutright DE (1976). A survey of factors possibly associated with cervical abrasion of tooth surfaces. *J Periodontol* 47(3)*:*148-154.

Rees JS, Jacobsen PH, Hickman J (1994). The elastic modulus of dentine determined by static and dynamic methods. *Clin Mater.*17(1):11-5.

Rees JS (2000). The prevalence of dentine hypersensitivity in general dental practice in the UK. *J Clin Periodontol* 27(11)*:*860-865.

Rees JS, Addy M (2002). A cross-sectional study of dentine hypersensitivity. *J Clin Periodontol* 29(11)*:*997-1003.

Rees JS, Jin LJ, Lam S, Kudanowska I, Vowles R (2003). The prevalence of dentine hypersensitivity in a hospital clinic population in Hong Kong. *J Dent* 31(7)*:*453-461.

Rees JS, Addy M (2004). A cross-sectional study of buccal cervical sensitivity in UK general dental practice and a summary review of prevalence studies. *Int J Dent Hyg* 2(2)*:*64-69.

Ren YF, Liu X, Fadel N, Malmstrom H, Barnes V, Xu T (2011). Preventive effects of dentifrice containing 5000ppm fluoride against dental erosion in situ. *J Dent* 39(10)*:*672-678.

Restarski JS, Gortner RA, Mccay CM (1945). The Effect of Acid Beverages Containing Fluorides Upon the Teeth of Rats and Puppies. *J of dent res* 24(3-4)*:*200-201.

Rice P (2012). SEM Charging Effects of SEM Images. SEM. In: NC Facility editor.

Richards LC, Kaidonis JA, Townsend GC (2003). A model for the prediction of tooth wear in individuals. *Aust Dent J* 48(4)*:*259-262.

Robb ND, Smith BG (1996). Chronic alcoholism: an important condition in the dentist-patient relationship. *J Dent* 24(1-2)*:*17-24.

Rodriguez JM, Austin RS, Bartlett DW (2012). In vivo measurements of tooth wear over 12 months. *Caries Res* 46(1)*:*9-15.

Ross MR (1961). Hypersensitive Teeth Effect of Strontium Chloride in a Compatible Dentifrice. *J of Period* 32(1)*:*49-&.

Sano H, Ciucchi B, Matthews WG, Pashley DH (1994). Tensile properties of mineralized and demineralized human and bovine dentin. *J Dent Res* 73(6)*:*1205-1211.

Sauro S, Watson TF, Thompson I (2010). Dentine desensitization induced by prophylactic and air-polishing procedures: an in vitro dentine permeability and confocal microscopy study. *J Dent* 38(5)*:*411-422.

Scaramucci T, Marquesmm, Soares-Geraldo D, Braga S, Sobral MA (2009). The influence of water temperature during toothbrushing on root dentine: an in vitro study. *Indian J Dent Res* 20(2)*:*185-189.

Scheie AA, Peterson FC (2008). Antimicrobials in caries control. In: Dental caries: The Disease and its Clinical Management. O Fejerskov and EAM Kidd editors. Oxford: Blackwell Munksgaard, pp. 263-277.

Scheutzel P (1996). Etiology of dental erosion - Intrinsic factors. *Euro J of Oral Sci* 104(2)*:*178-190.

Schiff T, Dotson M, Cohen S, De Vizio W, McCool J, Volpe A (1994). Efficacy of a dentifrice containing potassium nitrate, soluble pyrophosphate, PVM/MA copolymer, and sodium fluoride on dentinal hypersensitivity: a twelve-week clinical study. *J Clin Dent* 5 Spec No(87-92.

Schiff T, Bonta Y, Proskin HM, DeVizio W, Petrone M, Volpe AR (2000). Desensitizing efficacy of a new dentifrice containing 5.0% potassium nitrate and 0.454% stannous fluoride. *Am J Dent* 13(3)*:*111-115.

Schiff T, He T, Sagel L, Baker R (2006). Efficacy and safety of a novel stabilized stannous fluoride and sodium hexametaphosphate dentifrice for dentinal hypersensitivity. *J Contemp Dent Pract* 7(2)*:*1-8.

Schiff T, Delgado E, Zhang YP, DeVizio W, Cummins D, Mateo LR (2009). The clinical effect of a single direct topical application of a dentifrice containing 8.0% arginine, calcium carbonate, and 1450ppm fluoride on dentin hypersensitivity: the use of a cotton swab applicator versus the use of a fingertip. *J of Clin Dent* 20(4)*:*131-136.

Schilke R, Lisson JA, Bauss O, Geurtsen W (2000). Comparison of the number and diameter of dentinal tubules in human and bovine dentine by scanning electron microscopic investigation. *Arch Oral Biol* 45(5)*:*355-361.

Schlueter N, Ganss C, De Sanctis S, Klimek J (2005). Evaluation of a profilometrical method for monitoring erosive tooth wear. *Eur J Oral Sci* 113(6)*:*505-511.

Seligman DA, Pullinger AG, Solberg WK (1988). The prevalence of dental attrition and its association with factors of age, gender, occlusion, and TMJ symptomatology. *J Dent Res* 67(10)*:*1323-1333.

Seltzer S, Bender IB, Ziontz M (1963). The dynamics of pulp inflammation: correlations between diagnostic data and actual histologic findings in the pulp. *Oral Surg Oral Med Oral Pathol* 16 846-871 contd.

Seong J, Macdonald E, Newcombe RG, Davies M, Jones SB, Johnson S *et al.* (2012). In situ randomised trial to investigate the occluding properties of two desensitising toothpastes on dentine after subsequent acid challenge. *Clin Oral Investig*.

Serry FM (2011). Applications of Atomic Force Microscopy for Contact Lens Manufacturing.

Shah P, Razavi S, Bartlett DW (2009). The prevalence of cervical tooth wear in patients with bruxism and other causes of wear. *J Prosthodont* 18(5)*:*450-454.

Sharma S, Cross SE, Hsueh C, Wali RP, Stieg AZ, Gimzewski JK (2010). Nanocharacterization in dentistry. *Int J of mole sci* 11(6)*:*2523-2545.

Shellis RP, Finke M, Eisenburger M, Parker DM, Addy M (2005). Relationship between enamel erosion and liquid flow rate. *Eur J Oral Sci* 113(3)*:*232-238.

Smales FC, Mosedale RF, Floyd PM (1987). Policy for periodontal care. *Br Dent J* 163(5)*:*167-169.

Smith BG, Knight JK (1984a). An index for measuring the wear of teeth. *Br Dent J* 156(12)*:*435-438.

Smith BG, Knight JK (1984b). A comparison of patterns of tooth wear with aetiological factors. *Br Dent J* 157(1)*:*16-19.

Smith BG, Robb ND (1996). The prevalence of toothwear in 1007 dental patients. *J Oral Rehabil* 23(4)*:*232-239.

Smith WA, Marchan S, Rafeek RN (2008). The prevalence and severity of non-carious cervical lesions in a group of patients attending a university hospital in Trinidad. *J Oral Rehabil* 35(2)*:*128-134.

Statistical Bulletin: 2010-based National population projections, 23 November 2011.

Steele J, O'Sullivan, I (2011). Executive Summary Adult Dental Health Survey 2009. London: The Stationary Office

Steele JG, Walls AWG, Ayatollahi SMT, Murray JJ (1996). Major clinical findings from a dental survey of elderly people in three different English communities. *British Dental Journal* 180(1)*:*17-23.

Steiner-Oliveira C, Nobre-dos-Santos M, Zero DT, Eckert G, Hara AT (2010). Effect of a pulsed CO2 laser and fluoride on the prevention of enamel and dentine erosion. *Arch Oral Biol* 55(2)*:*127-133.

Suga N (2007). Metrology Handbook: The science of measurement.

Suge T, Kawasaki A, Ishikawa K, Matsuo T, Ebisu S (2005). Effects of pre- or post-application of calcium chloride on occluding ability of potassium oxalate for the treatment of dentin hypersensitivity. *Am J Dent* 18(2)*:*121-125.

Ten Cate AR (1998). Oral Histology: Development Structure and Function 5th ed Louis.

Ten Cate JM, Larsen MJ, Pearce E, Fejerskov O (2008). Chemical interactions between the tooth and oral fluids. In: Dental Caries: the disease and its clinical management. O Fejerskov and EAM Kidd editors. Oxford, Amres, Iowa: Blackwell Munksgaard.

Turssi CP, Messias DC, Hara AT, Hughes N, Garcia-Godoy F (2010). Brushing abrasion of dentin: effect of diluent and dilution rate of toothpaste. *Am J Dent* 23(5)*:*247-250.

Udoye CI (2006). Pattern and distribution of cervical dentine hypersensitivity in a Nigerian tertiary hospital. *Odonto-stomatologie tropicale= Tropical dental journal* 29(116)*:*19-22.

Van't Spijker A, Rodriguez JM, Kreulen CM, Bronkhorst EM, Bartlett DW, Creugers NHJ (2009). Prevalence of Tooth Wear in Adults. *Int J of Prosthodontics* 22(1)*:*35-42.

Van der Weijden FA, Campbell SL, Dorfer CE, Gonzalez-Cabezas C, Slot DE (2011). Safety of oscillating-rotating powered brushes compared to manual toothbrushes: a systematic review. *J Periodontol* 82(1)*:*5-24.

Vanuspong W, Eisenburger M, Addy M (2002). Cervical tooth wear and sensitivity: erosion, softening and rehardening of dentine; effects of pH, time and ultrasonication. *J Clin Periodontol* 29(4)*:*351-357.

Von Koppenfels RL, Kozak KM, Duschner H, White DJ, Goetz H, Taylor E *et al.* (2005). Stannous Floruide effects on Dentinal Tubules. IADR: *J of Dent Res.*

Walls AW, Steele JG (2001). Geriatric oral health issues in the United Kingdom. *Int Dent J* 51(3 Suppl)*:*183-187.

Wang Z, Sa Y, Sauro S, Chen H, Xing W, Ma X *et al.* (2010). Effect of desensitising toothpastes on dentinal tubule occlusion: a dentine permeability measurement and SEM in vitro study. *J Dent* 38(5)*:*400-410.

Watson TF (1991). Applications of confocal scanning optical microscopy to dentistry. *Br Dent J* 171(9)*:*287-291.

Watson TF, Boyde A (1991). Confocal light microscopic techniques for examining dental operative procedures and dental materials. A status report for the American Journal of Dentistry. *Am J Dent* 4(4)*:*193-200.

Watson TF, Petroll WM, Cavanagh HD, Jester JV (1992). In vivo confocal microscopy in clinical dental research: an initial appraisal. *J Dent* 20(6)*:*352-358.

Watson TF (1997). Fact and artefact in confocal microscopy. *Adv Dent Res* 11(4)*:*433-441.

Weber DF (1983). An improved technique for producing casts of the internal structure of hard tissues, including some observations on human dentine. *Arch Oral Biol* 28(9)*:*885-891.

West NX, Addy M, Jackson RJ, Ridge DB (1997). Dentine hypersensitivity and the placebo response. A comparison of the effect of strontium acetate, potassium nitrate and fluoride toothpastes. *Journal of Clinical Periodontology* 24(4)*:*209-215.

West NX, Hughes JA, Parker DM, Newcombe RG, Addy M (1999). Development and evaluation of a low erosive blackcurrant juice drink. 2. Comparison with a conventional blackcurrant juice drink and orange juice. *J Dent* 27(5)*:*341-344.

West NX, Hughes JA, Addy M (2000). Erosion of dentine and enamel in vitro by dietary acids: the effect of temperature, acid character, concentration and exposure time. *J Oral Rehabil* 27(10)*:*875-880.

West NX, Hughes JA, Addy M (2002). Dentine hypersensitivity: the effects of brushing toothpaste on etched and unetched dentine in vitro. *J Oral Rehabil* 29(2)*:*167-174.

West NX (2006). Dentine hypersensitivity. *Monogr Oral Sci* 20(173-189.

Wetton S, Hughes J, West N, Addy M (2006). Exposure time of enamel and dentine to saliva for protection against erosion: a study in vitro. *Caries Res* 40(3)*:*213-217.

Whelton H, Crowley E, O'Mullane DM, Guiney H, Cronin M, Flannery E *et al.* (2008). North South Survey of Children's Oral Health in Ireland 2002. Dublin: Department of Health.

White DJ, Lawless MA, Fatade A, Baig A, von Koppenfels R, Duschner H *et al.* (2007). Stannous fluoride/sodium hexametaphosphate dentifrice increases dentin resistance to tubule exposure in vitro. *J Clin Dent* 18(2)*:*55-59.

Whittacker DK (2000). Historical and forensic aspects of tooth wear. In: Tooth wear and Sensitivity- clinical advances in restorative dentistry. M Addy, G Emberry, R Edgar and R Orchardson editors. London: Martin Dunitz.

Whitworth JM (2010) Rational root canal treatment in practice. Quintessenz Verlag

WHO (version 2007). Diseases of oral cavity, salivary glands and jaws (K00-K14). International Classification of Diseases In: WH Organisation editor.

Wiegand A, Stock A, Attin R, Werner C, Attin T (2007). Impact of the acid flow rate on dentin erosion. *J Dent* 35(1)*:*21-27.

Wiegand A, Kuhn M, Sener B, Roos M, Attin T (2009). Abrasion of eroded dentin caused by toothpaste slurries of different abrasivity and toothbrushes of different filament diameter. *J Dent* 37(6)*:*480-484.

Williams RAD, Elliott JC (1989). Basic and applied dental biochemistry. 2nd ed. C Livingstone editor. Edingburgh.

Wu TS, Chang WJ, Hsu JC (2004). Effect of tip length and normal and lateral contact stiffness on the flexural vibration responses of atomic force microscope cantilevers. *Microelectron Eng* 71(1)*:*15-20.

Wulknitz P (1997). Cleaning power and abrasivity of European toothpastes. *Adv Dent Res* 11(4)*:*576-579.

Xhonga FA, Valdmanis S (1983). Geographic comparisons of the incidence of dental erosion: a two centre study. *J Oral Rehabil* 10(3)*:*269-277.

Yewe-Dyer M (1993). The definition of oral health. *Br Dent J* 174(7)*:*224-225.

Yoshiyama M, Suge T, Kawasaki A, Ebisu S (1996). Morphological characterization of tube-like structures in hypersensitive human radicular dentine. *J Dent* 24(1-2)*:*57-63.

Zapletalova Z, Kubinek R, Vujtek M, Novotny R (2004). Examination of dentin surface using AFM (our experience). *Acta Medica (Hradec Kralove) 2004;47(4):343-6*.

Zenith-International-Ltd (1997). The 1997 Sucralose Soft Drinks Report. Reading RG6 6BX, UK.

Zero DT (1996). Etiology of dental erosion--extrinsic factors. *Eur J Oral Sci* 104(2 (Pt 2))*:*162-177.

Zero DT, Lussi A (2005). Erosion--chemical and biological factors of importance to the dental practitioner. *Int Dent J* 55(4 Suppl 1)*:*285-290.

Zsiska M, Peterson BW, White DJ (2010). SnF2 Effects on Acid Resistance of HAP Mineral in Vitro. IADR: J of Dent Res.